# Fish fossils from Miocene palaeolakes in the East African Rift Valley in Kenya


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Vorgelegt von
Melanie Altner

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Erstgutachter: Prof. Dr. Bettina Reichenbacher

Zweitgutachter: Prof. Dr. Alexander Nützel

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## Statutory declaration and statement

I hereby confirm that my thesis entitled **"Fish fossils from Miocene palaeolakes in the East African Rift Valley, Kenya"**, is the result of my own original work. Furthermore, I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the Ludwig-Maximilians-University Munich.

# Abstract

Africa's modern-day freshwater fish fauna comprises more than 3000 species, many of them endemic, and is dominated by a few teleost lineages among which the Cyprinodontiformes and the Cichlidae are especially prominent. Even though members of both groups are used as model organisms in evolutionary studies, their evolutionary history is not yet fully understood. This is in part due to their scant fossil record.

The main purpose of this dissertation is to investigate the palaeodiversity of freshwater fishes on the African continent during the Miocene epoch, focusing particularly on the Lukeino and Ngorora Formations in the Central Kenya Rift. The material described in this study forms part of a collection consisting of about 650 articulated fish fossils, which were recovered from the upper Miocene Lukeino Formation and the middle–upper Miocene Ngorora Formation in the course of two field campaigns in 2013 and 2014. To provide a context for a better understanding of the new fossils of Cyprinodontiformes and Cichlidae, a comprehensive comparative dataset, including data on meristics, osteology, scale characters and otolith morphology, was assembled for extant representatives of both groups. The dataset for the Cichlidae includes all previously recognized lineages of African cichlids, and is the first of its kind designed to facilitate the phylogenetic placement of cichlid fossils. This new dataset was used to organize the taxonomic studies and to analyse the phylogenetic relationships of the fossils. The results presented here shed new light on the evolutionary history of both Cyprinodontiformes and Cichlidae, and provide new information on Miocene palaeoenvironments and hydrological networks in the Central Kenya Rift.

The upper Miocene Lukeino Formation has yielded numerous well-preserved cyprinodontiform fossils of an extinct lineage of the suborder Aplocheiloidei, which represent the first fossil record of this group. On the basis of the morphological study and the phylogenetic analysis, the new taxon was assigned to the new family †Kenyaichthyidae, the new genus †*Kenyaichthys* and the new species †*K. kipkechi* sp. nov. The specimens of †*K. kipkechi* show wide variation in their meristic counts and morphometric traits, which is comparable to that found in recent sympatric species with variable grades of hybridization. †*K. kipkechi* thus presumably represents a fossil species flock *in statu nascendi*. The phylogenetic analysis unexpectedly places †*K. kipkechi* in a sister relationship to the exclusively Neotropical family Rivulidae, a probable explanation might be lack of available synapomorphies for the Rivulidae, Nothobranchiidae and Aplocheilidae. Moreover, the comparison with the recent material revealed that previously proposed apomorphic characters concerning the neural and haemal spines of the preural vertebrae in the caudal skeleton should be revised. The scarcity of other typical freshwater fishes in the Lukeino Formation and the

close relationship of †*K. kipkechi* to the aplocheiloid families Nothobranchiidae and Rivulidae suggest a seasonally dry climate for the palaeoenvironment. Some recent members of the Nothobranchiidae and Rivulidae are known to withstand such harsh conditions due to their ability to produce desiccation-resistant eggs. The new taxon may perhaps have had a similar life cycle. In addition, deformities of the vertebral column are prominent in our sample and point to a strong influence of volcanic activity on the aquatic environment.

The study of the middle–upper Miocene Ngorora fish Lagerstätte uncovered two remarkable new fossil taxa of the family Cichlidae. The first is †*Protochromis pickfordi* nov. gen. nov. sp. Comparative osteological and meristic studies revealed that †*P. pickfordi* is closely related to the present-day tribes Ectodini and Limnochromini in Lake Tanganyika. This is further supported by a Principal Coordinates Analysis based on meristic data. Due to its unique character set, which includes a tripartite lateral line and a lacrimal with six lateral line tubules, it is suggested that †*P. pickfordi* belongs to a precursor lineage of the 'ancient Tanganyika mouth-brooders'. The presence of a Miocene precursor of Lake Tanganyika cichlids far outside the drainage area of the present-day Lake Tanganyika implies an ancient hydrological connection between the Central Kenya Rift and Lake Tanganyika, which supports the hypothesis that a significant portion of the genomic diversity of modern-day Lake Tanganyika cichlids is derived from riverine cichlids.

The second new taxon, †*Baringochromis* nov. gen., is based on the analysis of 335 well-preserved fossil specimens. Its unique combination of characters, most prominently one predorsal bone, six infraorbitals including a lacrimal with four or five lateral line tubules, a partially scaled suboperculum and a low number of anal and dorsal fin spines, puts it in an intermediate position between the almost pan-African Oreochromini and the exclusively East African Haplochromini. This placement is supported by a Principal Coordinates Analysis based on meristic data. The discovery of †*Baringochromis* therefore suggests a scenario in which precursor lineages of the present-day Oreochromini and Haplochromini were widely distributed throughout East Africa and underwent hybridization in rivers prior to the formation of the Rift Lakes.

Taken together, the data reveal that, due to their mosaic-like character sets, the new fossil cyprinodontiform and cichlid taxa cannot be confidently placed within available phylogenetic trees. This implies that the evolutionary history of these two freshwater fish groups cannot be described solely in terms of lineage splitting, and probably involved introgression and hybridization, as already suggested by studies based on molecular data for the Cichlidae. Moreover, the results highlight the value of fossil archives with high preservation quality, like the Lukeino Formation and the Ngorora fish Lagerstätte, for the understanding of past evolutionary processes.

# Zusammenfassung

Die heutige afrikanische Süßwasserfisch-Diversität mit ihren mehr als 3000 Arten ist großteils endemisch und wird von wenigen Linien der Teleostei dominiert an denen die Familie Cichlidae und die Ordnung der Cyprinodontiformes einen großen Anteil hat. Obgleich Mitglieder beider Gruppen in Evolutionsstudien als Modelorganismen fungieren, ist ihre Evolutionsgeschichte noch nicht völlig erforscht. Dies ist teilweise ihrem geringen Fossilbericht geschuldet.

Das Hauptziel dieser Dissertation ist es die Paläodiversität der afrikanischen Süßwasserfische im Miozän mit Fokus auf die Lukeino und Ngorora Formation im zentralkenianischen Grabenbruch zu untersuchen. Das hier beschriebene Material ist Teil einer 650 artikulierte Fischfossilien umfassenden Sammlung, welche im Rahmen zweier Feldstudien in Kenia in 2013 und 2014 entdeckt und aufgesammelt worden sind. Als Grundlage für die systematische Untersuchung der zu den Cyprinodontiformes und den Cichlidae gehörenden Fossilien wurde ein umfassendes Vergleichsmaterial, bestehend aus rezenten Vertretern beider Gruppen, zusammengestellt. Dieser Datensatz umfasst meristische und osteologische Merkmale sowie Daten zur Schuppen- und Otolithenmorphologie. Der Datensatz für die Cichliden enthält alle bisher bekannten Linien afrikanischer Cichliden; mit diesem Datensatz steht nun erstmals eine solide Basis zur systematischen Einordnung fossiler Cichliden zur Verfügung. Auf dieser Grundlage erfolgten die taxonomischen Studien und die Untersuchung der phylogenetischen Beziehungen der Fossilien. Die hier vorgestellten Ergebnisse leisten einen Beitrag zum Verständnis der Evolutionsgeschichte der Cyprinodontiformes und der Cichlidae und bieten außerdem neue Informationen zur Rekonstruktion der Paläoumwelt und ehemaligen Gewässernetze im zentralkenianischen Grabenbruch.

Die obermiozäne Lukeino Formation lieferte zahlreiche sehr gut erhaltene Fossilien einer ausgestorbenen Linie der Unterordnung Aplocheiloidei, welche gleichzeitig die erste fossile Überlieferung dieser Gruppe darstellt. Basierend auf der morphologischen Untersuchung und der phylogenetischen Analyse wurde die neue Familie †Kenyaichthyidae sowie die neuen Gattung †*Kenyaichthys* und die neue Art †*K. kipkechi* eingeführt. Die fossilen Individuen von †*K. kipkechi* zeigen eine große Variabilität bezüglich ihrer meristischen und morphometrischen Merkmale, vergleichbar mit rezenten sympatrischen Arten die sich noch miteinander paaren und teilweise fortpflanzungsfähige Hybride erzeugen. †*K. kipkechi* kann daher als ein im Entstehen begriffener fossiler Artenschwarm (*in statu nascendi*) interpretiert werden. Die phylogenetische Analyse stellt †*K. kipkechi* wider Erwarten in eine Schwestergruppenverhältnis zu den ausschließlich in der Neuen Welt (Südamerika) verbreiteten Familie der Rivulidae; eine mögliche Erklärung könnte die geringen Anzahl bisher bekannter Synapomorphien sein. Darüber hinaus zeigte die Analyse des Vergleichsmaterials, dass bisher beschriebene apomorphe Merkmale bezüglich der neuralen und hämalen Dornfortsätze der präuralen Wirbel des Schwanzflossenskelettes nicht uneingeschränkt anwendbar sind und der Revision bedürfen. Die Seltenheit anderer typischer Süßwasserfische in der Lukeino Formation und das nahe Verwandtschaftsverhältnis zwischen †*K. kipkechi* und den aplocheiloiden Familien Nothobranchiidae und Rivulidae deutet auf eine von saisonal aridem Klima beeinflusste Paläoumwelt hin. Einige rezente Vertreter der

Nothobranchiidae und Rivulidae sind dafür bekannt dass sie solchen extremen Bedingungen widerstehen können indem sie Austrocknungs-resistente Eier produzieren. Das neue Taxon könnte durch einen vergleichbaren Lebenszyklus an die ariden Phasen angepasst gewesen sein. Außerdem weisen die bei manchen fossilen Individuen auftretenden Deformationen der Wirbelsäule auf vulkanische Beeinflussung des Gewässers hin.

Darüber hinaus ergab die Studie der Ngorora Fisch-Lagerstätte die Überlieferung zweier neuer Taxa aus der Familie der Cichlidae. Das erste ist †*Protochromis pickfordi* nov. gen. nov. sp. Vergleichende osteologische und meristische Studien konnten zeigen dass eine nahe Verwandtschaft von †*P. pickfordi* zu den heute ausschließlich im Tanganjika-See verbreiteten Tribes der Ectodini and Limnochromini besteht. Diese Annahme wird zusätzlich durch eine Hauptkoordinaten-Analyse auf der Grundlage meristischer Daten unterstützt. Auf Grund seiner einzigartigen Merkmalskombination, welche eine dreigeteilte Seitenlinie und ein Lacrimale mit sechs Seitenlinienkanälchen umfasst, ist anzunehmen dass †*P. pickfordi* zu einer Vorgängerlinie der sogenannten 'ancient Tanganyika mouth-brooders' gehört. Die Anwesenheit einer miozänen Vorläuferlinie der rezenten Tanganjika-Cichliden weit außerhalb des Einzugsbereichs des heutigen Tanganjika-Sees impliziert ein ehemaliges Gewässernetz zwischen dem zentralkenianischen Grabenbruch und dem Tanganjika-See. Dies wiederum unterstützt die Hypothese dass ein signifikanter Anteil der genetischen Diversität der heutigen Tanganjika-Cichliden aus Introgression und Hybridisierung mit in Flüssen verbreiteten Cichliden hervorgegangen ist.

Das zweite Taxon, †*Baringochromis*, ist anhand von 335 gut erhaltenen fossilen Individuen dokumentiert. Auf Grund seiner einzigartigen Merkmalskombination, wie die Anwesenheit eines Prädorsalia, sechs Infraorbitalia einschließlich eines Lacrimals mit vier oder fünf Seitenlinienkanälchen, eines teilweise beschuppten Suboperculums sowie einer geringen Anzahl von Flossenstacheln in der After- und Rückenflosse, wird eine intermediäre Position zwischen den fast pan-afrikanisch verbreiteten Oreochromini und den ausschließlich ostafrikanischen Haplochromini angenommen. Dies wird zusätzlich durch eine Hauptkoordinaten-Analyse auf der Grundlage meristischer Daten unterstützt. Dies lässt ein Szenario vermuten, in welchem Vorgängerlinien der Oreochromini und Haplochromini in Ostafrika weit verbreitet und sich untereinander gepaart haben bevor der afrikanische Grabenbruch die ehemals zusammenhängenden Gewässernetze unterbrochen hat und die großen Seen entstanden sind.

Abschließend lässt sich sagen, dass, aufgrund der einzigartigen Merkmalskombinationen, die neuen fossilen Taxa sowohl der Cyprinodontiformes wie auch der Cichlidae nicht eindeutig innerhalb der verfügbaren phylogenetischen Stammbäume platziert werden können. Daraus lässt sich folgern dass die Evolutionsgeschichte dieser Süßwasserfische nicht allein durch unidirektionales Aufspalten von Linien erklärt werden kann, und dass Prozesse wie Introgression und Hybridisierung eine bedeutende Rolle bei der Diversifikation gespielt haben können, was auch neueste molekulare Studien für die Cichliden postuliert haben. Schließlich verdeutlichen die Ergebnisse die Bedeutung von sedimentären Archiven mit hoher Qualität der fossilen Überlieferung, wie die der Lukeino Formation und der Ngorora Fisch-Lagerstätte, für das Verständnis evolutionärer Prozesse.

# Contents

# 1. Introduction

## 1.1 General aspects

Fishes are the most species-rich group of vertebrates on Earth, making up more than 50% of total vertebrate species diversity (Nelson, 2006; Helfman et al., 2009). They display an extraordinary amount of diversity in body shape, habitat and behaviour, which often makes it difficult to understand their phylogenetic relationships and evolutionary history (Nelson, 2006; Helfman et al., 2009). Based on their primary mode of skeletal biomineralization, fishes can be divided into two major lineages: the bony fishes (Osteichthyes) and the cartilaginous fishes (Chondrichthyes) (Nelson, 2006; Helfman et al., 2009). The bony fishes comprise the ray-finned fishes (Actinopterygii) and the lobe-finned fish (Sarcopterygii), with the latter giving rise to the tetrapods (e.g. Ahlberg et al., 2005; Carroll, 2005; Ahlberg and Clack, 2006; Shubin et al., 2006; Miller et al., 2007). The Actinopterygii encompass the modern bony fishes (Teleostei), which is the most diverse clade of living fishes. Among Teleostei, the species-rich Percomorpha have received considerable scientific attention, because the phylogenetic relationships of this group remain to be fully explored (see Near et al., 2013). Molecular studies have shown that Percomorpha can be divided into nine supraordinal groups, which have so far not been recovered on the basis of anatomical features (Betancur-R. et al., 2013; Near et al., 2013). Among the new clades are the Ovalentaria, which include the family Cichlidae and the order Cyprinodontiformes (Betancur-R. et al., 2013; Near et al., 2013; Betancur-R et al., 2014).

## 1.2 Cyprinodontiformes and Cichlidae

Cyprinodontiformes, commonly named killifish, top minnows or tooth carps are a large and diverse group of fishes comprising more than 1300 species (Eschmeyer and Fong, 2016). The members of this group are usually slender-bodied and small, ranging from three to five centimetres in total length and their fins are exclusively soft-rayed (Parenti, 1981; Costa, 1998).

The 1400 species (Kolm et al., 2006b; Fitzsimmons and Watanabe, 2010) of the Cichlidae so far recognised display a wide range of body shapes, and sizes vary between approximately 3 cm in *Neolamprologus multifasciatus* (see Schradin and Lamprecht, 2002) and 99 cm in *Cichla temensis* (see IGFA, 2001). They can easily be recognized by their external features such as the single nostril on each side of the head, the divided lateral line, and the presence of

a spiny and a soft-rayed part in the dorsal and anal fins (Skelton, 2001; Kullander, 2003; Nelson, 2006).

Cyprinodontiformes and Cichlidae are of great scientific significance and have been used as model organisms for various biological studies (e.g. Herrera and Jagadeeswaran, 2004; Kocher, 2004; Burnett et al., 2007; Duvernell et al., 2008; Maderbacher et al., 2008; Dorn et al., 2011; Edenbrow and Croft, 2011; Juntti et al., 2013; Dorn et al., 2014; Xu et al., 2014; Kratochwil et al., 2015). Moreover, their peculiar color patterns and diverse mating and breeding behaviours make both groups popular as aquarium fish (e.g. Parenti, 1981; Costa, 1998; Hemdal, 2003; Liew et al., 2012; Oconner, 2012).

### 1.2.1 Adaptations to extreme environments

Members of both Cyprinodontiformes and Cichlidae have been reported to be well adapted to challenging environmental conditions, such as low oxygen concentration, salinity, alkalinity and extreme temperature. Frequently cited examples among killifish are the euryhaline and eurytherm species of *Aphanius* (Wildekamp, 1993; Hrbek and Meyer, 2003; Reichenbacher et al., 2009a; Reichenbacher et al., 2009b). Examples among the Cichlidae include the euryhaline species of *Oreochromis* (Watanabe et al., 1985; Avella et al., 1993; Uchida et al., 2000) and the species of *Alcolapia* and *Oreochromis amphimelas* in the highly alkaline Lakes Magadi, Natron, Manyara, Eyasi, Kitangiri and Singida (Tichy and Seegers, 1999; Bayona, 2006; and references cited therein; Ford et al., 2015; Kavembe, 2015).

### 1.2.2 Specializations

The unparalleled range of biological adaptations to diverse and sometimes inhospitable habitats found among Cyprinodontiformes and Cichlidae is accompanied by remarkable specialisations of their morphology, life history and behaviour. Unlike the vast majority of bony fishes (including cichlids), which are oviparous, the Cyprinodontiformes have evolved the ability to give birth to live young (=live-bearing killifishes) (Meyer and Lydeard, 1993; Blackburn, 2005; and references cited therein) on at least three different occasions (in Goodeidae, Anablepidae and Poeciliidae). In contrast, the Cichlidae have evolved different forms of parental care, with numerous transitions from substrate guarding to mouth-brooding and from uniparental to biparental care (Goodwin et al., 1998; Klett and Meyer, 2002; Kolm et al., 2006a; Sefc, 2011; and references cited therein).

A peculiar reproductive strategy is known from some aplocheiloid Cyprinodontiformes that live in temporary ponds in South America and Africa. Before the onset of desiccation of the

pond and the die-off of the adults in early summer, these species produce desiccation-resistant eggs that remain buried in the sediment, enabling the embryos to start a new life cycle as soon as the ponds are refilled (Wourms, 1972; Haas, 1976; Murphy and Collier, 1997; Hrbek and Larson, 1999; Wildekamp, 2004; Berois et al., 2012; and references cited therein).

### 1.2.3 Speciation

One of the major reasons why Cyprinodontiformes and Cichlidae are studied by evolutionary biologists is their propensity for rapid adaptive radiation and sympatric speciation. Examples among the Cyprinodontiformes are the species of *Aphanius* on the Iranian plateau (e.g. Esmaeili et al. 2014), the species flocks of *Cyprinodon* in the Laguna Chichancanab in Mexico (Strecker, 2006; and references cited therein) and the littoral species of *Orestias* in Lake Titicaca in Peru (Parenti, 1984; Northcote, 2000; and references cited therein). Some of the species that form a flock can be differentiated solely based on the head shape, which is most probably due to trophic adaptation (Northcote, 2000; Horstkotte and Strecker, 2005; Horstkotte and Plath, 2008; and references cited therein).

The most extraordinary examples of adaptive radiations are known for the Cichlidae in the African Great Lakes (Lake Tanganyika, Malawi and Victoria), each of which harbours hundreds of closely related cichlid species (Salzburger and Meyer, 2004; Meyer, 2005; Loh et al., 2013; and references cited therein), while the so-called ‘superflock’ of Lake Victoria and neighbouring Lakes Albert, Edward, George, Kyoga and Kivu is thought to have arisen within less than 100,000 years ago (Seehausen et al., 2003; Verheyen et al., 2003; Salzburger and Meyer, 2004; Meyer, 2005; and references cited therein). In contrast to previous ideas, recent studies have provided evidence for the hypothesis that at least some of the genomic diversity present in each of these species flocks is derived from riverine cichlids. This in turn implies that part of the speciation process had already occurred prior to the colonization of the African Great Lakes (Seehausen et al., 2003; Genner et al., 2015; Weiss et al., 2015; and references cited therein).

### 1.2.4 Phylogenetic relationships

The phylogenetic affinities of the Cyprinodontiformes are well understood based on both morphological and molecular data (Costa, 1998, 2004). Two suborders are recognized i.e. the Cyprinodontoidei and the Aplocheiloidei (Parenti, 1981), which comprise seven and three families, respectively (Costa, 2004). Within the three families of the Aplocheiloidei, the Madagascan/South Asian aplocheiloids (Aplocheilidae) are the most basal taxa and sister to

an African-South American dichotomy (Rivulidae+Nothobranchiidae, Murphy and Collier, 1997).

The four major subfamilies of the Cichlidae (Ptychochrominae, Etroplinae, Pseudocrenilabrinae, Cichlinae) are clearly defined based on molecular data (e.g. Sparks, 2004; Sparks and Smith, 2004; Dunz and Schliewen, 2013). According to these studies the Etroplinae (restricted to Madagascar, India and Sri Lanka) represent the most plesiomorphic lineage, followed by the Ptychochrominae (restricted to Madagascar) and the two sister clades Cichlinae (restricted to South America) and Pseudocrenilabrinae (restricted to Africa) (Sparks, 2004; Sparks and Smith, 2004). In contrast, phylogenetic analyses based on morphological data have failed to reconstruct a monophyletic African lineage but place the African genus *Heterochromis* either close to the Etroplinae or among basal Cichlinae (Stiassny, 1991; Kullander, 1998; Farias et al., 1999; and references cited therein). Below the subfamily level, the cichlids are assigned to so-called 'tribes' based on morphological similarity. These tribes are designated with the ending '-ini' (e.g. Poll, 1986; Takahashi 2003; Meyer et a. 2014), whereas informal group names use the ending '-ines' (see Schwarzer, 2011). There have been many attempts to further elucidate the internal systematics of the family, based on morphological characters such as the pharyngeal apophysis, the scales, the lateral line foramina on the head and other delicate structures (e.g. Regan, 1920, 1922; Cichocki, 1976; Greenwood, 1978; Lippitsch, 1990; Stiassny, 1991; Lippitsch, 1992; Casciotta and Arratia, 1993; Lippitsch, 1993, 1995, 1997; Kullander, 1998; Lippitsch, 1998; Takahashi, 2003b, a). However, these efforts have largely failed to reconstruct the phylogenetic affinities at the level of tribes and focussed only on some of the major lineages.

Meanwhile, molecular data have shown the South American cichlids to comprise seven tribes (Smith et al., 2008), whereas the African cichlids can be separated into 27 lineages (if the Orthochromines and Tropheini are counted as separate lineages). Among the African cichlids, the most basal lineages include the Heterochromini, Tylochromini, Chromidotilapiines, Hemichromines and Pelmatochromines, followed by the highly diverse Haplotilapiines comprising 22 lineages (Schwarzer et al., 2009; Dunz and Schliewen, 2013; Loh et al., 2013; Weiss et al., 2015; and references cited therein). The monophyly of the Haplotilapiines is additionally supported by the synapomorphy 'presence of tricuspid inner row dentition on the oral jaws' (Schliewen and Stiassny, 2003). Among the Haplotilapiines, the East African Radiation (EAR), encompassing 13 lineages, is the most speciose clade within the African cichlids, and includes the radiations in the East African Lakes Tanganyika, Malawi and

Victoria (Stiassny et al., 2007; Schwarzer et al., 2009; Dunz and Schliewen, 2013; Loh et al., 2013; Weiss et al., 2015; and references cited therein).

### 1.2.5 Biogeography and age

Both the Cichlidae and the Cyprinodontiformes are extremely diverse in species and show an almost worldwide circumtropical distribution, with the exception of Australia (Skelton, 2001; Chakrabarty, 2004; Collier et al., 2009). This present-day distribution has led to two hotly debated hypotheses relating to their historical biogeography and age: i) the vicariance hypothesis (Parenti, 1981; Parker and Kornfield, 1995; Murphy and Collier, 1997; 1999 and references cited therein) and ii) the dispersal theory (Lundberg, 1993; Briggs, 2003 and references cited therein). According to the first, both groups were evenly distributed throughout Gondwana during the Cretaceous and their contemporary distribution is linked to the fragmentation of the super-continent (Parker and Kornfield, 1995; Murphy and Collier, 1997 and references cited therein). According to the dispersalists however, the Cyprinodontiformes and Cichlidae are thought to have spread out from a so-called 'centre of origin', by crossing marine waters during the middle or late Cretaceous. Support for this theory is derived from reports of species which are tolerant to saltwater, like many groups among the killifishes (e.g. most of the cyprinodonts, some aplocheiloids), (Lundberg, 1993; Briggs, 2003 and references cited therein) and species of Cichlidae like *Tilapia*, *Sarotherodon* and *Oreochromis* (Murray, 2001a; and references cited therein). Due to the absence of fossils older than the Oligocene (in the case of killifish, see Gaudant, 2012) or Eocene (in the case of cichlids, see Murray, 2000, 2001b), the issue has not yet been resolved (see discussions in Murphy and Collier, 1997; Murray, 2001a; Chakrabarty, 2004; Friedman et al., 2013).

## 1.3 Significance of this study

The fossil record is an indispensable source of information for a better understanding of the evolutionary history of the Cyprinodontiformes and Cichlidae. Moreover, fossils represent excellent proxy indicators for the reconstruction of biogeographical relationships and hydrological networks, palaeoenvironments and palaeoclimate (e.g. Lévêque, 1997; Stewart, 2001; Otero et al., 2009; Otero, 2010). However, the fossil record of freshwater fishes is especially scanty and mostly consists of isolated bones, scales and teeth (e.g. Stewart, 2001; Otero et al., 2008; Otero et al., 2009; Otero, 2010; Pinton et al., 2011), which makes a confident systematic assignment at the species, genus or even tribe level difficult. It is therefore particularly important to explore archives that yield high-quality fish fossils, i.e.

complete and well-preserved specimens, as is the case for the Lukeino and Ngorora Formations (see Rasmussen et al., 2015). An additional interesting facet of their study for the broader community is the palaeoenvironmental reconstruction of the middle-late Miocene in Central Kenya, because this is the time when the ancestors of modern hominids evolved (e.g. Senut et al., 2001; Jacobs, 2002; Sawada et al., 2002; Bonnefille, 2010).

## 1.4 Aims and outline of the dissertation

### 1.4.1 Overall goal

The aim of this study was to provide new information on the palaeodiversity of freshwater fishes on the African continent during the Miocene epoch in order to facilitate understanding of the evolutionary history of the living forms.

### 1.4.2 Overview of manuscripts

Chapter 2 is a taxonomic study of 169 cyprinodontiform fossils from the upper Miocene Lukeino Formation. It describes the first fossil record of the suborder Aplocheiloidei. A comparative osteological examination and a phylogenetic analysis based on 72 osteological characters support the erection of the new family †Kenyaichthyidae, the new genus †*Kenyaichthys* and the new species †*K. kipkechi* sp. nov. Moreover, the phylogenetic analysis unexpectedly places †*K. kipkechi* in sister relationship to the exclusively Neotropical Rivulidae, which adds new information regarding character evolution within the families of the Aplocheiloidei and indicates a biased distribution of synapomorphies relative to the Nothobranchiidae. The specimens not only display a unique combination of characters but also high levels of variation in their osteological and meristic characters, and are thus interpreted as a species flock *in statu nascendi*. Moreover, a comparative analysis including 47 specimens of 10 extant species (belonging to three families) raises doubts concerning previously suggested apomorphic characters of the Cyprinodontoidei and Aplocheiloidei. The marked preponderance of †*K. kipkechi* and the corresponding scarcity of other typical freshwater fishes in our sample supports the previous assumption of a seasonally dry climate for the late Miocene of Eastern Africa, to which †*K. kipkechi* seems to have been well adapted. Indeed, it may have had an annual lifestyle like the members of recent aplocheiloids.

In Chapter 3, a unique fossil cichlid specimen from the middle to upper Miocene Ngorora Formation is examined and the new genus and species †*Protochromis pickfordi* is introduced for it. It shows a mosaic character set intermediate between the Ectodini and Limnochromini, which both belong to the so-called 'ancient Tanganyika mouth-brooders'. It is therefore assumed to represent a member of a precursor lineage leading to the modern Lake Tanganyika cichlids. Moreover, the discovery of this fossil in the Central Kenya Rift (far away from present-day Lake Tanganyika) indicates an ancient hydrological connection between the Central Kenya Rift and Lake Tanganyika, and furthermore supports the hypothesis that hybridization with riverine cichlids played a major role in the diversification of Lake Tanganyika cichlids.

In Chapter 4 a taxonomic study based on 335 cichlid specimens from the Ngorora Formation, of the site "Waril" is presented. The new taxon †*Baringochromis* and four new species, †*B. senutae*, †*B. sonyii*, †*B. stellae* and †*B. davidae* are introduced. These four species show differences in their head shape and oral tooth morphology, and presumably represented a species flock. As in †*Protochromis*, a mosaic character set is also present in †*Baringochromis.* In the case of †*Baringochromis*, the specimens investigated seem to be "intermediate" between the widely distributed Oreochromini and the East African Haplochromini. Like †*Protochromis*, the taxon †*Baringochromis*, which can be interpreted as a precursor lineage of Oreochromini and Haplochromini, is providing further support for the scenario of widely distributed riverine precursor lineages prior to the formation of the present-day Rift lakes.

Author contributions

Chapter 2: **Melanie Altner**, Bettina Reichenbacher: †Kenyaichthyidae fam. nov. and †*Kenyaichthys* gen. nov. – First Record of a Fossil Aplocheiloid Killifish (Teleostei, Cyprinodontiformes)

MA designed and conducted the analyses, acquired, analysed, interpreted the data, and drafted the manuscript. MA and BR contributed to discussions and the final manuscript.

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Chapter 3: **Melanie Altner**, Ulrich Schliewen, Bettina Reichenbacher: †*Protochromis pickfordi* gen. et sp. nov. from the upper Miocene – a precursor lineage of modern Lake Tanganyika cichlids?

MA designed and conducted the analyses, acquired, analysed, interpreted the data, and drafted the manuscript. US provided necessary tools and specimens. All authors contributed to data interpretation, discussions and the final manuscript.
Manuscript submitted to the Journal of Vertebrate Paleontology.

Chapter 4: **Melanie Altner**, Bettina Reichenbacher: Exceptionally well-preserved fossil cichlids from the Miocene Ngorora Fish Lagerstätte of Central Kenya with the description of †*Baringochromis* gen. nov.
MA designed and conducted the analyses, acquired, analysed, interpreted the data, and drafted the manuscript. MA and BR contributed to discussions and the final manuscript.
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# Results

The following subchapters are presented in the form of scientific articles.

## 2. †Kenyaichthyidae fam. nov. and †*Kenyaichthys* gen. nov. – First Record of a Fossil Aplocheiloid Killifish (Teleostei, Cyprinodontiformes)

Melanie Altner*, Bettina Reichenbacher
Department of Earth- and Environmental Sciences, Palaeontology & Geobiology, Ludwig-Maximilians-University, Munich, Germany

**Abstract**
The extant Cyprinodontiformes (killifishes) with their two suborders Cyprinodontoidei and Aplocheiloidei represent a diverse and well-studied group of fishes. However, their fossil record is comparatively sparse and has so far yielded members of the Cyprinodontoidei only. Here we report on cyprinodontiform fossils from the upper Miocene Lukeino Formation in the Tugen Hills of the Central Rift Valley of Kenya, which represent the first fossil record of an aplocheiloid killifish. A total of 169 specimens - mostly extraordinarily well preserved - and a sample of ten extant cyprinodontiform species were studied on the basis of morphometrics, meristics and osteology. A phylogenetic analysis using PAUP was also conducted for the fossils. Both the osteological data and the phylogenetic analysis provide strong evidence for the assignment of the fossils to the Aplocheiloidei, and justify the definition of the new family †Kenyaichthyidae, the new genus †*Kenyaichthys* and the new species †*K. kipkechi* sp. nov. The phylogenetic analysis unexpectedly places †Kenyaichthys gen. nov. in a sister relationship to the Rivulidae (a purely Neotropical group), a probable explanation might be lack of available synapomorphies for the Rivulidae, Nothobranchiidae and Aplocheilidae. The specimens of †*K. kipkechi* sp. nov. show several polymorphic characters and large overlap in meristic traits, which justifies their interpretation as a species flock in statu nascendi. Patterns of variation in neural and haemal spine dimensions in the caudal vertebrae of †*Kenyaichthys* gen. nov. and the extant species studied indicate that some previously suggested synapomorphies of the Cyprinodontoidei and Aplocheiloidei need to be revised.

## 2.1. Introduction

The extant order Cyprinodontiformes contains about 1,120 species (Costa, 2012) and displays a virtually worldwide circumtropical distribution, with the exception of Australia (Collier et al., 2009). According to Parenti (Parenti, 1981) the order consists of two suborders, the Cyprinodontoidei and the Aplocheiloidei, with a total of ten families. The families of the Cyprinodontoidei include the Cyprinodontidae (United States, Central and South America, the West Indies, Africa, Europe, and Asia), Poeciliidae (United States, Central and South America, and Africa), Fundulidae (United States, Central America, and Canada), Profundulidae (Central America), Anablepidae (southern Mexico to southern South America), Goodeidae (United States), and Valenciidae (Mediterranean region) (Parker and Kornfield, 1995; Nelson, 2006; Froese and Pauly, 2014). The families of the Aplocheiloidei can be separated into the Neotropical Rivulidae (South America) and the Old World Nothobranchiidae and Aplocheilidae (Africa, Madagascar, India, and South Asia) (Collier et al., 2009).

Given the huge diversity of the living Cyprinodontiformes, their fossil record is comparatively poor and is so far restricted to the Cyprinodontoidei. The highest species diversity is known for the extinct †*Prolebias* SAUVAGE, 1874, from the Oligocene and Miocene of Europe and Asia, which has recently been identified as a paraphylum and now includes several additional genera (Costa, 2012; Gaudant, 2012). Another extinct genus known from the Miocene of Europe is †*Aphanolebias* Reichenbacher and Gaudant, 2003 (Reichenbacher and Kowalke, 2009). In addition, a few fossil species of the extant genus *Aphanius* NARDO, 1827 have been reported from the Miocene and Pliocene of Europe and Asia (Gaudant, 1993; Cubells et al., 1994; Gaudant, 2002, 2011; Rückert-Ülkümen et al., 2002; Carnevale et al., 2006; Vasilyan et al., 2009). Furthermore, a single fossil species of *Cyprinodon* LACÉPÈDE, 1803 from the late Pliocene and several species of *Fundulus* LACÉPÈDE, 1803 from the middle Miocene to early Pleistocene have been reported from the United States (see review by Smith (1981); Livingston and Dattilo (2004)). Fossil species of the Anablepidae such as †*Carrionellus* WHITE, 1927 from the early Miocene (Costa, 2011) and Jenynsia GÜNTHER, 1866 from the late middle Pleistocene (Bogan et al., 2009) have been reported from South America (Arratia and Cione, 1996), whereas *Empetrichthys* GILBERT, 1893 has been reported from the Pliocene of the United States (Uyeno and Miller, 1962). Additionally, several fossil taxa of the Goodeidae such as *Alloophorus*, *Goodea*,

*Chapalichthys*, *Ameca*, *Girardinichthys*, *Xenotoca*, and †*Tapatia* have been reported from the late Miocene to late Pliocene of North America (see review by Miller and Smith (1986); Guzman and Polaco (2009)). Undetermined species of the Poeciliidae have been described from the Eocene Lumbrera Formation (Porto et al., 1982; Arratia and Cione, 1993; Cione and Baez, 2007; Garcia et al., 2012) and the Miocene San José and Rio Salí Formations of Argentina (Cione, 1986; Arratia and Cione, 1993; Cione and Baez, 2007), and *Poeciliopsis* is known from the Pleistocene of Mexico (Guzman and Polaco, 2009). In contrast to this comparatively rich record of Cyprinodontoidei, no fossil species of the Aplocheiloidei have yet been described.

Most authors argue that the origin of the Cyprinodontiformes dates to the Cretaceous at least (see Briggs, 2003). Some authors assume that they had an ancient Gondwana-wide distribution and that their present-day distribution is linked to the break-up of Gondwana (vicariance hypothesis) (Parker and Kornfield, 1995; Murphy and Collier, 1997, 1999). Others argue that the Cyprinodontiformes originated in South America and that their radiation is linked to dispersal in the middle or late Cretaceous (Lundberg, 1993; Briggs, 2003). However, previously reported records of Cyprinodontiformes from strata older than Oligocene are scarce and some of them are now regarded as doubtful. Thus the identification of fossil specimens from the upper Cretaceous Molino Formation of Bolivia, South America as cf. Cyprinodontiformes (Gayet, 1992; Gayet et al., 1992) is not supported in Gayet and Meunier (1998). Fossil scales of a putative species of †*Cyprinodon* (*C*. (?) *primulus*) from the upper Paleocene to lower Eocene Maíz Gordo Formation of Argentina described by Cockerell (1936) have been re-interpreted as Cyprinodontiformes indet (Arratia and Cione, 1993; Cione and Baez, 2007) or Poeciliidae indet (Marchio and Piller, 2013). However, these scales do appear to represent the first secure fossil record of Cyprinodontiformes, indicating that the order is of late Paleocene (56–59 Ma) age at least.

The objective of this study is to describe newly discovered fossils of killifishes from the upper Miocene Lukeino Formation in Kenya. †Kenyaichthyidae nov. fam., †*Kenyaichthys* nov. gen., and †*K . kipkechi* sp. nov. are introduced. †*Kenyaichthys* is the first fossil record of the Aplocheiloidei.

### 2.1.1 Stratigraphic and sedimentological context

Study area. The study area is located in the Tugen Hills in the Central Rift Valley of Kenya (Fig. 1A–1B). One of the most complete Neogene successions in Africa is found here, with exposures consisting of sedimentary strata that alternate with volcanic rocks of middle Miocene to Pleistocene age (Pickford et al., 2009). The fish-bearing diatomaceous shales belong to the Lukeino Formation, which is about 110 m thick and of late Miocene (5.7–6 Ma) age (see Hill et al., 1985; Hill and Drake, 1986). Among the fossils previously described from the Lukeino Formation are freshwater faunal elements (gastropods, bivalves, crocodiles, hippos and turtles) and terrestrial remains such as dicotyledonous leaves (Bamford et al., 2013), as well as one of the earliest hominids known (Pickford, 1975; Senut et al., 2001; Pickford et al., 2009).

The fossils described here come from Inoswa Kamelon (0°45'43.71"N 35°50'7.98"E; locality 2/215N in Pickford et al. (2009) near "a small hillside east of the Bartabwa-Yatya road" (Pickford et al., 2009 page 77) and from Koibochepkweny (00°50'10.6"N 35°54'29.2"E; locality 2/222 in Pickford et al. (2009) about 3 km north of

Inoswa Kamelon, "east of the Yatya-Bartabwa road" (Pickford et al., 2009 page 80). All fossils were collected by a research team led by M. Pickford and B. Senut (both Muséum National d´Histoire Naturelle Paris) in 2004–2005, in collaboration with the members of the local Orrorin-Community-Organisation.

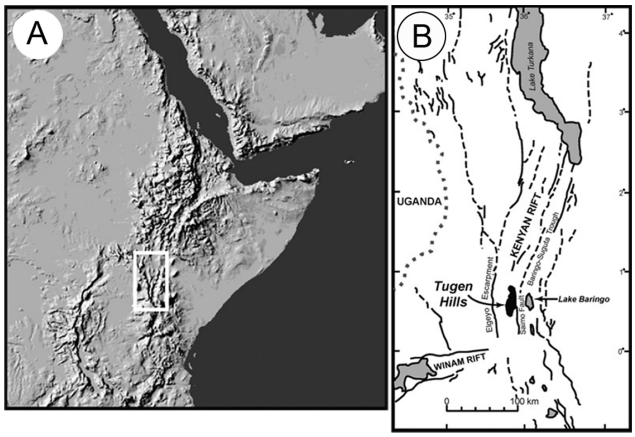


**Figure 1. Geographic overview of the East African Rift System (EARS) and the study site** (Reprinted from (Kingston et al., 2002)). **A** Map of East Africa with the location of the EARS; **B** Detail of the EARS with the location of the Tugen Hills.

## 2.2. Materials and Methods

### 2.2.1. Fossil Material

Inoswa Kamelon yielded 164 fish specimens, almost half of which (72) are complete. Koibochepkweny yielded five complete specimens. All fossils have been deposited in the

Museum in Kipsaraman, Kenya, which is affiliated with the National Museum in Nairobi. Fossils are labelled with the prefix BAR (for Baringo) and the following numbers: 1141´04–1237´04, 1324´04, 1325´04, 1192a/b´05, 1203a/b´05, 1204´05, 1209a/b´05, and 1218a/b´05. Silicone casts of 49 selected specimens are kept in the Bavarian State Collection for Palaeontology and Geology, Munich, Germany (BSPG) under the numbers BSPG 2013 XXV 1–49. All extinct taxa are indicated with †.

### 2.2.2. Comparative Material Examined

Suborder Cyprinodontoidei, Family Cyprinodontidae:

• *Aphanius sophiae* (Heckel, 1847), Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz, Iran (ZM-CBSU) 281, 283, 284, 6171, 6193, 8296, 8401, 10883, 10884, 10962, C227, C295, C316, Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz, Iran (ZM-CBSUZG) 177, 178, 183 –185, 188 (17 cleared and stained specimens (c&s) and two x-rayed specimens (xr) from the Kor Basin, SW Iran; see (Gholami et al., 2013)),

• *Aphanius farsicus* (Teimori, Esmaeili, Reichenbacher, 2011), ZM-CBSUZG 1, 8, 13, 140, 141, 142 (six xr from the Marharlu Basin, SW Iran; see (Gholami et al., 2013)),

• *Aphanius arakensis* (Teimori, Esmaeili, Gholami, Zarei, Reichenbacher, 2012), ZM-CBSUZG 350, 352, 354, 356, 359, 361 (two c&s and four xr from the Namek Basin, SW Iran; see Gholami et al. (2013)),

• *Aphanius mesopotamicus* (Coad, 2009), ZM-CBSUZG 362, 363, 364, 365 (four c&s from the Karun Basin, SW Iran; see Gholami et al., (2013)).

Suborder Aplocheiloidei, Family Aplocheilidae:

• *Pachypanchax playfairii* (GÜNTHER, 1866), Musée Royal de l´Afrique Centrale, Tervuren, Belgium (MRAC) P.188937-188938 (two c&s from Les Canelles, Mahé Sud, Seychelles).

Suborder Aplocheiloidei, Family Nothobranchiidae:

• Aphyose*mion castaneum* Myers, 1924, MRAC 91-080-P-0063-0064 (two c&s from the Masendula River, Haut-Zaire, Zaire);

• *Epiplatys sexfasciatus* Gill, 1862, MRAC 92-052-P-0512-0513 (two c&s from a side channel of the Sombreiro River at the new Ahoada bridge, Nigeria);

• *Foerschichthys flavipinnis* (Meinken, 1932), MRAC 91-001-P-0378-0379 (two c&s from Taylor Creek, Biseni, Niger Delta, Nigeria);

• *Fundulopanchax sjoestedti* (Lönnberg, 1895), MRAC 91-100-P-0050-0051 (two c&s from drying swampforest waters 2 –3 km east of Kaiama, on East-West road near the turn-off to Kalama village, Nigeria);
• *Nothobranchius orthonotus* (Peters, 1844), MRAC A4-039-P-0133-0134 (two c&s from a site on the road from Nicoladala to Caia Ferry, Mozambique).

### 2.2.3. Methods

Obscuring sediment matrix was removed from fossil specimens under a stereomicroscope, using dissecting needles, and peels based on 49 selected specimens were produced by applying dyed silicone in thin coats. Extant specimens (see Comparative Material) were cleared and stained for cartilage and bone following the protocol of Taylor and Van Dyke (1985).

Osteological, meristic and morphometric characters of the fossil and extant specimens were studied under a stereomicroscope equipped with a digital camera. The standard length (SL) and total length (TL) were measured based on digital images using ImageJ version 1.49a (Rasband, 1997–2014) and recorded to the nearest 0.1 mm. All other measurements were recorded to the nearest

0.01 mm. Morphometric and meristic characters follow Holcik (1989) (Fig. 2A), apart from dorsal and anal fin ray counts, which included every detectable ray, whether supported by a proximal radial (pterygiophore) or not. In the case of individuals that were represented by part and counterpart, both parts were considered in the character analysis, while only one value (the maximal value) of the respective measurement or count was included in the statistical analyses.

All measurements were standardized based on the standard length. Data from the literature were taken into account in the interpretation of osteological characters (Parenti, 1981; Costa, 1998a, b, 2004, 2012).

The widths of the spines of the preural vertebrae (PU) PU2– PU5 were measured and, as spine ratios are considered to be important for phylogenetic analysis within Cyprinodontiformes, ratios for PU2/PU4, PU2/PU5, and PU3/PU5 were calculated based on both neural and haemal spines (see Tables S4 and S8). Measurements of spines attached to PU2, PU3, and PU4 were based on the width of the most distal part of the respective neural or haemal spine (Fig. 2B1 and S1); in the case that the distal tip of a spine was obscured by

caudal fin rays, its width was measured just before these rays. Measurements of spines attached to PU5 were based on the maximal width in the distal third of the respective neural or haemal spine (calculation of the distal third was based on the entire spine length including the arch). If spines were split or duplicated, we measured the broader of the two spines. For comparison with extant material, we determined PU2/PU4 and PU2/PU5 haemal spine ratios of 1.0 and < 2.0 as "slightly wider" and ratios of 2.0 as "distinctively wider".

Phylogenetic reconstructions were performed using PAUP version 4.0b10 (Swofford, 2003), characters with unknown state were coded as question marks; all character states were treated as unordered and unweighted. Selection of outgroups (Atherinomorpha and Beloniformes) followed Costa (Costa, 1998a). Statistical analyses were performed using SPSS version 21.0 (IBM Corp, 2012). All necessary permits were obtained for the described study, which complied with all relevant regulations. The research clearance permit was obtained from the National Council for Science and Technology.

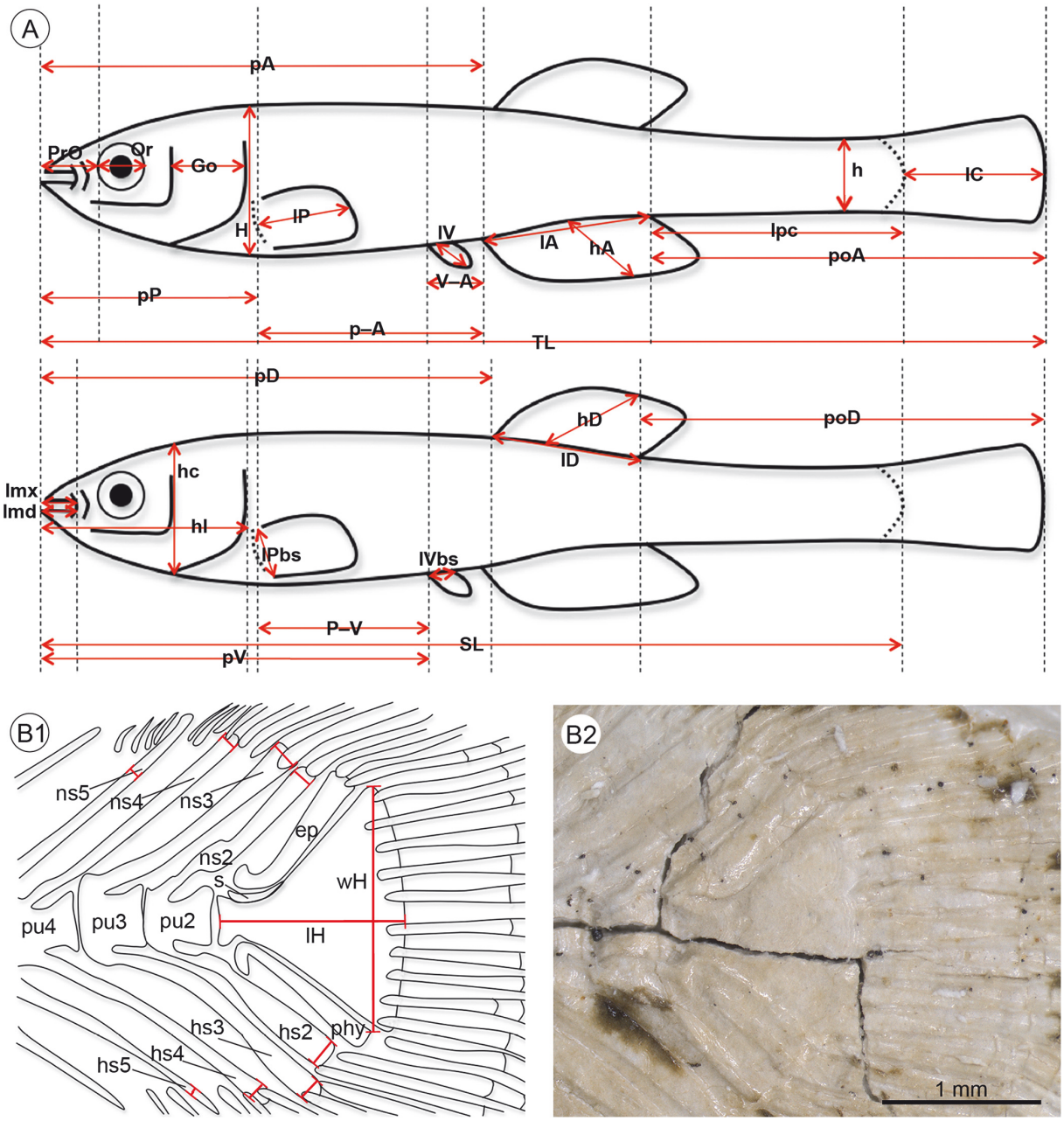


**Figure 2. Schematic drawings indicating measurements used in this study. A**, morphometric parameters; **B1–B2** measurements of hypural plate length and width and measurements of the spine widths on the caudal skeleton of †*K. kipkechi* sp. nov. (paratype 1200´04); note that the proximal part of the parhypural (with an anteroventral projection) does not overlap the terminal centrum, left lateral view. Abbreviations: **ep**, epural; **Go**, gill opening; **H**, maximum body depth; **h**, minimum body depth; **hA**, depth of anal fin; **hc**, head depth; **hD**, depth of dorsal fin; **hs2–5**, haemal spine of preural vertebrae 2–5; **lA**, length of anal fin base; **lc**, length of head; **lC**, length of caudal fin; **lD**, length of dorsal fin base; **lmd**, lower jaw length, i.e. distance from anteriormost point of lower jaw symphysis to posteriormost margin of mandibular joint; **lH**, length of hypural plate; **lmx**, upper jaw length, i.e. distance between anteriormost point of premaxillary and posteriormost point of maxillary; **lP**, length of pectoral fin; **lPbs**, length of pectoral fin base; **lpc**, length of caudal peduncle; **lV**, length of pelvic fin; **lVbs**, length of pelvic fin base; **ns2–5**, neural spine of preural vertebrae 2–5; **Or**, eye diameter; **pA**, preanal distance; **P–A**, distance between pectoral fin base and anal fin base; **pD**, predorsal distance; **phy**, parhypural; **poA**, postanal distance, i.e. from posterior end of anal fin to end of caudal fin rays; **poD**, postdorsal distance, i.e. from posterior end of dorsal fin to end of caudal fin rays; **pP**, prepectoral distance; **prO**, preorbital distance; **pu2–4**, preural vertebrae 2–4; **pV**, prepelvic distance; **P–V**, distance between pectoral fin base and pelvic fin base; **s**, stegural; SL, standard length; **TL**, total length; **V–A**, distance between pelvic fin base and anal fin base; **wH**, width of hypural plate.

## 2.3. Results

### 2.3.1 Systematic Palaeontology

Order Cyprinodontiformes Berg, 1940

Suborder Aplocheiloidei Parenti, 1981

Family †Kenyaichthyidae fam. nov.

**Type Genus**. †*Kenyaichthys*, gen. nov.

Diagnosis. Differs from other known families of the Aplocheiloidei in the combination of the following characters: first vertebra with distinctive neural spine vs. neural spine of first vertebra absent in Aplocheilidae; first vertebra with two long and narrow neuroapophyses of equal length and width lateral to the narrow neural spine vs. first vertebra with two short lateral neuroapophyses and broad neural spine in some Rivulidae and all Nothobranchiidae vs. first vertebra with distinctive neural spine and neuroapophyses absent in some Rivulidae vs. first vertebra with median neural spine and neuroapophyses absent in remaining Rivulidae; pelvic girdle with laterally pointed process vs. no laterally pointed process in those Rivulidae, in which this character has been examined; five or six preural vertebrae vs. four or five preural vertebrae in all Rivulidae, Nothobranchiidae and Aplocheilidae; rod-shaped epipleural ribs vs. bifid epipleural ribs in Nothobranchiidae and some Rivulidae; long ventral portion of autopalatinum reaching the quadratum vs. short autopalatinum not reaching quadratum in Rivulidae; robust, L-shaped preoperculum vs. thin, C-shaped preoperculum in Rivulidae; lateral rim of frontals not reduced vs. lateral rim of frontals reduced in Rivulidae; posterior tip of the ascending process of the premaxilla not medially curved vs. posterior tip of the ascending process of the premaxilla medially curved in Aplocheilidae and Nothobranchiidae.

†*Kenyaichthys* gen. nov.

(Figs. 3–12)

**Type and only known species.** †*Kenyaichthys kipkechi* sp. nov.

**Etymology.** Named for the country in which the specimens were found (Kenya), and ichthys (Greek) used to refer to fishes; gender feminine

**Diagnosis.** As for the family.

†*Kenyaichthys kipkechi* sp. nov.

(Figs. 3–12)

**Holotype.** 1209a/b´05.

**Referred Specimens.** 77 specimens, designated by the prefix BAR and the following numbers [(1)–(2) indicate individual specimens when more than one specimen is preserved on the same slab, and R indicates specimens on the rear side of the slab; "/" indicates presence of part and counterpart]: 1142´04, 1144/1146´04, 1145´04, 1147´04, 1148(1)´04, 1149´04, 1150´04, 1151/1152´04, 1153´04, 1154a/b´04, 1155´04, 1156´04, 1157(1)/1158(1)´04, 1159a(1)/b(1)´04, 1159a(2)/b(2)´04, 1160a/1161b´04, 1160b/1161a´04, 1162´04, 1163a(1)/b(2)´04, 1163a(2)/b (1)´04, 1164a/b´04, 1165a/b´04, 1166a´04, 1166b´04, 1167´04, 1168´04, 1170´04, 1171´04, 1172´04,1174´04, 1175´04, 1176a/b´04, 1177´04, 1178(1)´04, 1180(1)´04, 1181(1)´04, 1181(2)/1183(1)´04, 1182´04, 1184(1)´04, 1185/1186´04, 1187´04–1189´04, 1190´04, 1192a/b´05, 1192´04, 1193´04, 1194´04, 1198a/b´04, 1199a/b´04, 1200´04, 1202´04, 1203a/b´05, 1204´05, 1204´04, 1206(1)/1211´04, 1209´04, 1213(1)´04, 1215(1)´04, 1217a(1)/b(1)´04, 1218´04, 1218a/b´05,1219(1)´04, 1220(1)´04, 1220R´04, 1221(1)´04, 1227(1)´04, 1228(1)/1237R(1)´04, 1233/1234(1)´04, 1234(2)´04, 1234R´04, 1236(1)´04, 1237(1)´04, 1237(2)´04, 1324´04, 1325 ´04. In addition, 92 further specimens are tentatively assigned as †K. cf. kipkechi because of incomplete or fragmentary preservation (see Table S3).

**Age.** Late Miocene, about 5.7–6 Ma.

**Locality.** Tugen Hills, Inoswa Kamelon (72 specimens) and Koibochepkweny (5 specimens), Lukeino Formation, Kenya.

**Etymology**. Named for Joseph Kipkech, Kenya, in recognition of his long-time devoted commitment to the development of education and science in Baringo County.

**Diagnosis.** As for the family.

**Description.** Small fishes, ranging in size from 22 to 40 mm total length (TL), and 20 to 36 mm standard length (SL) (see Tables 1 and S1for measurements). Most specimens are preserved in lateral view, indicating that the body is elongate and laterally compressed. Body height reaches a maximum between the posterior margin of the head and the origin of the pectoral fin, and ranges from16–28% of SL. The minimum body height, ranging from 6–12% SL, lies at the middle of the caudal peduncle. Head length ranges from 25–34% SL; the lateral profile from the snout to the occiput is asymmetrical, with a weakly rising long anterior part, and a curving short posterior part (Fig. 3). The snout is slightly pointed, with the lower jaw

slightly longer than the upper (superior mouth) (see Tables 1 and S1and Fig. 3). The caudal peduncle is long and elongate (22–30% SL), and reveals a slightly concave ventral and dorsal profile (Fig. 3A1). The caudal fin ranges in size from 8 to 17% SL and is rounded to truncate (Fig. 3A7). Cycloid scales are visible on the whole body and parts of the head (operculum, preoperculum, frontals), but not on the fins, with the exception of a few scales on the caudal fin base (Fig. 4A3).

**Table 1. Morphometric characters** (given in mm and in %of SL) for †*Kenyaichthys kipkechi* sp. nov. based on the holotype (1209a/b´04) and all other specimens. n= number of specimens.

| Characters | Holotype | | All other specimens (mean values SD and ranges) | |
|---|---|---|---|---|
| Morphometrics | mm | % SL | mm | % SL |
| Total length (73) | 39.70 | 111.20 | 30.09 ± 3.20 (22.10–38.50) | 112.50 ± 2.02 (107.09–115.91) |
| Standard length (77) | 35.70 | – | 26.83 ± 2.80 (20.40–33.40) | – |
| Ratio of head length to head depth (68) | 1,45 | – | 1.53 ± 0.15 (1.27–1.97) | – |
| Length of head (76) | 10.46 | 29.30 | 7.92 ± 1.05 (5.73–10.85) | 29.61 ± 2.28 (24.73–33.94) |
| Head depth (68) | 7.19 | 20.14 | 5.22 ± 0.80 (3.60–7.05) | 19.52 ± 2.38 (15.16–24.41) |
| Eye diameter (72) | 2.78 | 7.79 | 2.32 ± 0.38 (1.41–3.26) | 8.65 ± 1.09 (5.72–12.21) |
| Gill opening (74) | 2.73 | 7.65 | 2.29 ± 0.35 (1.36–3.34) | 8.56 ± 0.98 (6.05–10.31) |
| Preorbital distance (72) | 3.53 | 9.89 | 2.35 ± 0.55 (1.07–3.89) | 8.50 ± 1.67 (4.04–12.44) |
| Length of dorsal fin base (70) | 3.93 | 11.01 | 3.51 ± 0.55 (2.49–4.89) | 13.15 ± 1.52 (9.83–16.16) |
| Depth of dorsal fin (58) | – | – | 2.67 ± 0.51 (1.25–3.63) | 10.02 ± 1.65 (6.13–14.83) |
| Length of anal fin base (73) | 4.92 | 13.78 | 4.01 ± 0.60 (1.91–5.65) | 14.97 ± 1.82 (9.05–19.49) |
| Depth of anal fin (62) | – | – | 2.52 ± 0.48 (1.13–3.33) | 9.38 ± 1.55 (5.42–12.87) |
| Length of pectoral fin (60) | 2.52 | 7.06 | 2.45 ± 0.57 (1.09–3.70) | 9.04 ± 1.92 (4.61–13.31) |
| Length of pectoral fin base (67) | 1.48 | 4.15 | 1.15 ± 0.29 (0.56–1.97) | 4.29 ± 0.94 (2.17–7.16) |
| Length of pelvic fin (65) | 1.13 | 3.17 | 1.26 ± 0.30 (0.46–1.92) | 4.69 ± 1.00 (2.35–6.96) |
| Length of pelvic fin base (66) | 0.24 | 0.67 | 0.25 ± 0.06 (0.13–0.44) | 0.93 ± 0.24 (0.48–1.72) |
| Length of caudal fin (71) | 3.94 | 11.04 | 3.59 ± 0.49 (1.99–4.71) | 13.50 ± 1.63 (8.21–16.77) |
| Minimum body depth (72) | 2.51 | 7.03 | 2.30 ± 0.38 (1.62–3.25) | 8.60 ± 1.12 (6.41–11.82) |
| Maximum body depth (61) | 7.35 | 20.59 | 5.56 ± 0.84 (3.80–7.67) | 20.60 ± 2.54 (16.42–27.89) |
| Predorsal distance (75) | 21.68 | 60.73 | 15.63 ± 1.70 (11.43–19.38) | 58.38 ± 2.12 (50.04–65.05) |
| Preanal distance (77) | 22.07 | 61.82 | 15.99 ± 1.96 (11.54–20.81) | 59.52 ± 2.53 (52.91–66.05) |
| Postdorsal distance (66) | 13.29 | 37.23 | 11.03 ± 1.25 (7.87–13.76) | 41.56 ± 2.75 (34.56–49.80) |
| Postanal distance (67) | 12.65 | 35.43 | 10.47 ± 1.21 (7.49–13.62) | 39.32 ± 3.18 (31.15–53.83) |
| Length of caudal peduncle (74) | 9.09 | 25.46 | 6.98 ± 0.75 (5.33–8.69) | 26.10 ± 1.60 (21.51–29.72) |
| Distance between Pectoral-Anal fins (72) | 10.34 | 28.96 | 7.06 ± 0.93 (5.15–9.74) | 26.24 ± 2.40 (20.36–31.92) |
| Distance between Pelvic-Anal fins (70) | 3.93 | 11.01 | 2.30 ± 0.46 (1.16–3.26) | 8.59 ± 1.52 (4.61–11.91) |
| Distance between Pectoral-Pelvic fins (69) | 6.38 | 17.87 | 4.74 ± 0.67 (3.17–6.19) | 17.71 ± 1.89 (12.93–21.25) |
| Prepelvic distance (71) | 18.29 | 51.23 | 13.70 ± 1.79 (9.44–18.69) | 51.04 ± 2.70 (45.17–57.69) |
| Prepectoral distance (72) | 11.89 | 33.31 | 8.99 ± 1.37 (6.32–13.42) | 33.28 ± 2.54 (28.26–40.18) |
| Lower jaw length (64) | 3.80 | 10.64 | 2.81 ± 0.40 (1.95–3.87) | 10.44 ± 1.30 (7.53–14.05) |
| Upper jaw length (58) | 3.68 | 10.31 | 2.65 ± 0.42 (1.75–3.56) | 9.81 ± 1.15 (7.17–13.00) |
| Hypural plate length (77) | 1.39 | 3.89 | 0.78 ± 0.35 (0.11–1.41) | 2.85 ± 1.15 (0.48–4.85) |
| Hypural plate width (74) | 1.50 | 4.20 | 0.91 ± 0.39 (0.30–1.71) | 3.34 ± 1.27 (1.14–5.60) |

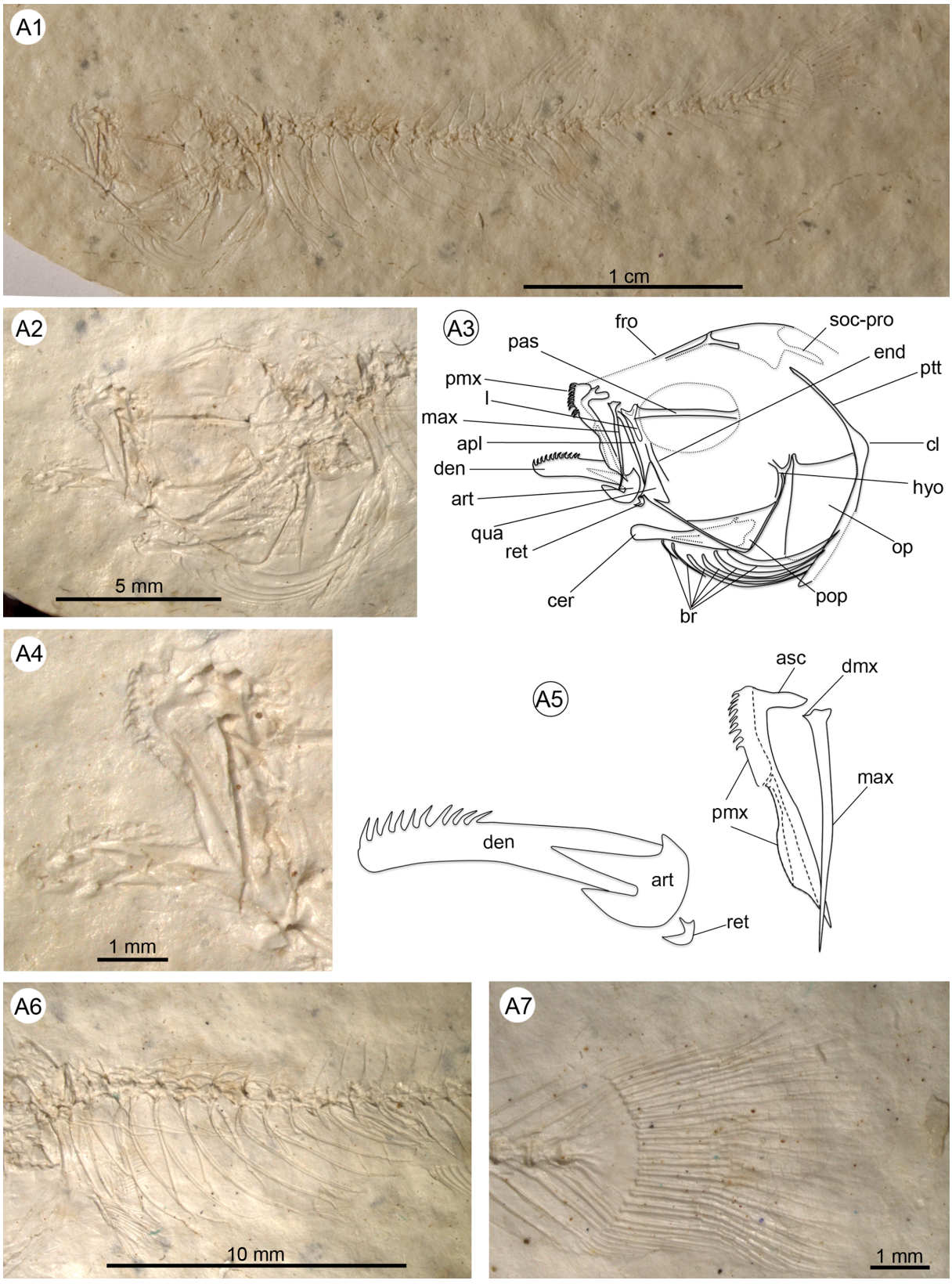


**Figure 3: Anatomical details of †*Kenyaichthys* nov. gen**. (holotype 1209a´05, †*K. kipkechi*), left lateral view. **A1** General overview (photograph by Dr. W. Altner); **A2–A3** Close up of the head and pectoral girdle (the lacrimal is from the counterpart and mirrored for clarity); **A4–A5** Close-up of lower and upper jaw; **A6** Detail of abdominal part showing vertebrae, pleural ribs and epipleural ribs; **A7** Truncate to rounded caudal fin. **Abbreviations**: **apl**, autopalatinum; **art**, anguloarticular; **asc**, premaxillary ascending process; **br**, branchiostegal rays; **cer**, ceratohyal; **cl**, cleithrum; **den**, dentary; **dmx**, dorsal maxillary process; **end**, endopterygoid; **fro**, frontal; **hyo**, hyomandibula; **l**, lacrimal; **max**, maxilla; **op**, operculum; **pas**, parasphenoid; **pmx**, premaxilla; **pop**, preoperculum; **ptt**, posttemporal; **qua**, quadratum; **ret**, retroarticular; **soc-pro**, supraoccipital process.

**Neurocranium.** The frontals are large and rectangular (Fig. 4B). The parietal is not clearly assignable in any of the specimens. The lacrimal is best recognizable in the three counterparts of the paratypes 1203b´05, 1209b´05 and 1218a´05; it is laterally reduced, i.e. longer than wide and Y-shaped (Fig. 3A3).

It is unclear whether the lacrimal is twisted. The supraoccipital is pentagonal with two parallel horn-like processes at the posterior margin. The parasphenoid is long and elongate, and crosses the orbit approximately at its middle (Fig. 3A2–3A3).

**Branchiocranium.** The shape of the dentary is elongate; its upper limb is probably as deep as the lower limb (Fig. 3A4–3A5). A single row of slightly recurved conical teeth is present on the anterior half of the dentary. The anguloarticular has a median process that is pointed and clearly longer than the ventral process, which is transversely expanded and truncated (Fig. 3A4–3A5). The coronoid process of the anguloarticular is pointed and small, and displays a slight concavity at the junction with the median process; the retroarticular is short (Fig. 3A4–3A5).

The toothless maxilla is long and slender and has three tiny processes at its anterior end. The first of these is anteriorly directed and represents the dorsal process; the two other structures probably comprise the ventral process (Fig. 3A4–3A5).

The premaxilla and maxilla are of nearly equal length (pmx: 7.0– 11.8% SL, mean 8.7±1.1% vs. mx: 7.0– 10.1% SL, mean 8.2±0.9%; see Table S2). The premaxilla is considerably wider and bears teeth on the anterior third of the bone. The straight alveolar arm shows an anterior expansion and a straight posterior border (Fig. 3A4–3A5). The ascending process is prominent, but relatively short, i.e. about one-sixth to one-fifth of the alveolar arm length. As far as can be discerned, the ascending process is not medially curved (Figs. 3A4–3A5 and 4A1–4C).

The autopalatinum is long, slender, but clearly thicker than the maxilla and overlaps the upper portion of the quadratum; its head is bent anteriorly (Figs. 3A2–3A4 and 4A1–4A2). The quadratum is triangular in shape, with an approximate angle of 110° between its dorsal and anterior margin and a long, almost straight or slightly concave, posterior margin (Figs. 3A2–3A3 and 4A1–4A2). The endopterygoid is slender with the dorsal margin reduced and not in contact with the metapterygoid. The symplectic is as long and as wide as the metapterygoid (Fig. 4A1–4A2).

The operculum is triangular in shape, with an angle of about 90° between its dorsal and anterior margin (Fig. 4A1–4A2). Its posterior margin is slightly rounded and the dorsal articular process is extended and pointed. A rounded and half-moon-shaped suboperculum is recognizable only in some disarticulated specimens. The preoperculum is robust and L-shaped (Figs. 3A2–3A3 and 4A1–4A2).

The ceratohyal is long and distally widened, and displays six branchiostegal rays. These show a stepwise increase in width from the first two rays (which are slender) posteriorly (Figs. 3A2–3A3 and 4A1–4A2). Notably, no scales appear in the region of the branchiostegal rays, whereas the adjacent regions (preoperculum, operculum, pectoral girdle) are covered with scales.

The gill arches are not clearly recognizable, but one specimen (1212a/b´04) does show a pharyngobranchial tooth-plate that bears multiple rows of molariform teeth.

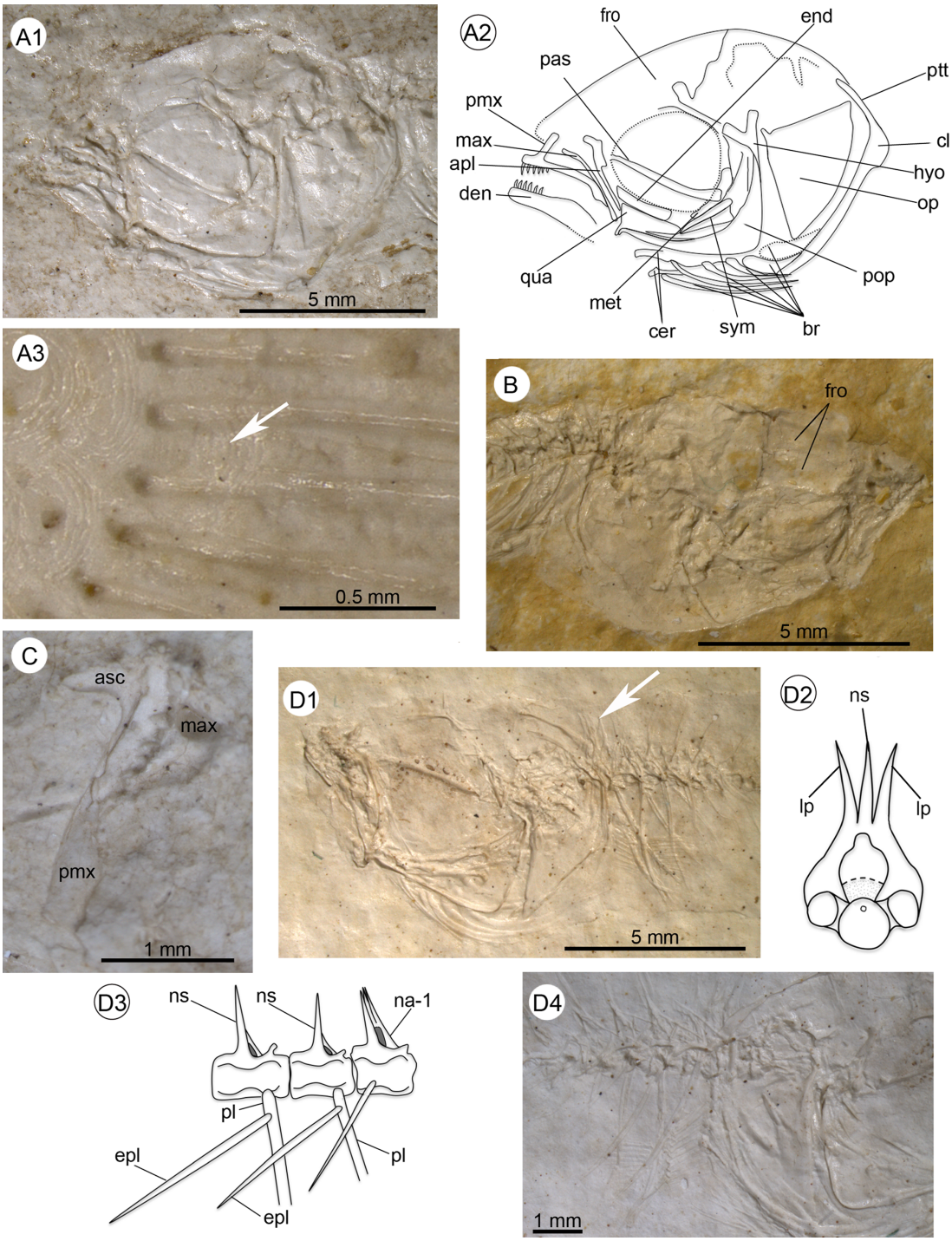


**Figure 4. Anatomical details of †*Kenyaichthys*. A1–A3** Anatomical details of †*K. kipkechi* sp. nov. (paratype 1237R(1)′04): **A1–A2** Head and pectoral girdle, left lateral view; **A3** Caudal fin rays covered with a single scale (arrow), left lateral view; **B** Head of †*K. kipkechi* sp. nov. (paratype 1160b′04), right dorsolateral view; **C** Disarticulated premaxilla and maxilla of †*Kenyaichthys* cf. *kipkechi* (1226a(1)′04), right lateral view; **D1–D4** Anatomical details of †*K. kipkechi* (paratype 1209a/b′05): **D1** Head and anterior part of body (1192a′05), arrow indicates lateral processes and spine of the first vertebra, left lateral view; **D2** Schematic reconstruction of the first vertebra (paratype 1192a/b′05), anterior view; **D3** Reconstruction of vertebrae 1–3 with pleural ribs and rodshaped epipleural ribs (1192b′05), left lateral view; **D4** Head and anterior part of body (1192b′05) with epipleural ribs on vertebrae 1–5, left lateral view. **Abbreviations**: **apl**, autopalatinum; **br**, branchiostegal rays; **cer**, ceratohyal; **cl**, cleithrum; **den**, dentary; **end**, endopterygoid; **epl**, epipleural rib; **fro**, frontal; **hyo**, hyomandibula; **lp**, lateral process; **max**, maxilla; **met**, metapterygoid; **na-1**, neural arch of first vertebra; **ns**, neural spine; **op**, operculum; **pas**, parasphenoid; **pl**, pleural rib; **pmx**, premaxilla; **pop**, preoperculum; **ptt**, posttemporal; **qua**, quadratum; **sym**, symplectic.

**Vertebral column.** In 50% of the specimens, where the vertebral column is preserved until the end of the dorsal fin, the vertebral column is straight to slightly curved, whereas in the remainder, the abdominal part of the vertebral column displays a hunchback-like curve (Fig. 5A–5D). The total number of vertebrae varies from 29 to 33, of which 11– 15 are abdominal (i.e. lie anterior to the first anal pterygiophore) and 17–21 are caudal (including the terminal centrum; see Tables 2 and S3). The first vertebra bears a distinctive median neural spine (recognizable in 36 specimens see Table S6).

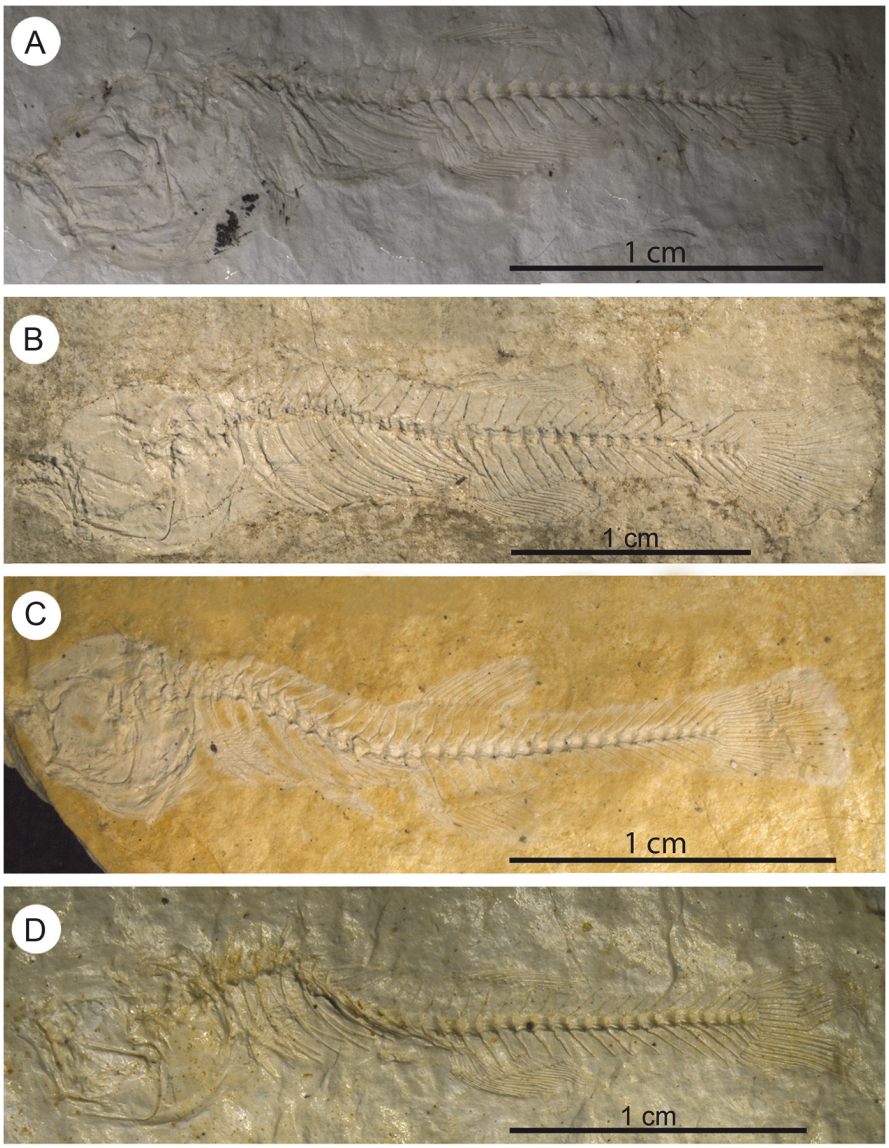


**Figure 5. General view of four specimens of †*K. kipkechi* sp. nov. showing the varying extent of curvature of the vertebral column**. **A**, straight (paratype 1146´04, mirrored); **B**, almost straight (paratype 1228(1)´04, mirrored); **C**, strongly curved (paratype 1168´04); **D**, strongly curved (paratype 1206(1)´04).

Three equally long and narrow neural processes appear in specimen 1192a/b´05 (Fig. 4D1–4D4); the median process probably corresponds to the median neural spine, whereas the two lateral processes represent neuroapophyses. The neural spines of the abdominal vertebrae are approximately upright, long, and reach almost to the dorsal margin of the body (Figs. 3A1, 3A7, 4D1 and 4D4). Small prezygapophyses are present on the abdominal, but not on the caudal vertebrae. Thick lateral parapophyses for connection to the ribs appear on the

abdominal vertebrae. Eight to 13 pairs of long ribs, starting at the second vertebra and extending to the ventral margin of the abdominal cavity, are present (Table S3); the first up to nine pairs of ribs bears long, thin, rod-shaped epipleurals (Figs. 3A6 and 4D3–4D4).

The neural and haemal spines of the anterior caudal vertebrae are upright, whereas those of the following ones are posteriorly inclined. All spines of the caudal vertebrae are long and almost reach the dorsal (ventral) border of the body (see also the description of the caudal axial skeleton).

**Girdles.** The pectoral girdle displays a prominent cleithrum with an extended dorso-posterior portion, and a comparatively slender ventral portion. The posttemporal is long, thin and unforked (Fig. 6A1–6A2); a supracleithrum is not clearly recognizable. It is possible that the two bones were fused. A postcleithrum is also not evident. The coracoid is long, and probably incompletely preserved; its posterior region is slightly indented below the fourth radial. The radials are robust and cubical in shape and all are approximately of the same size (Fig. 6A1–6A2). The outline of the scapula is not clearly recognizable.

The pelvic bones are relatively long and triangular. An anteromedial process is lacking. The medial process and the ischial process are minute (Fig. 6B1–6B2). A peculiar feature is the presence of long and pointed lateral processes.

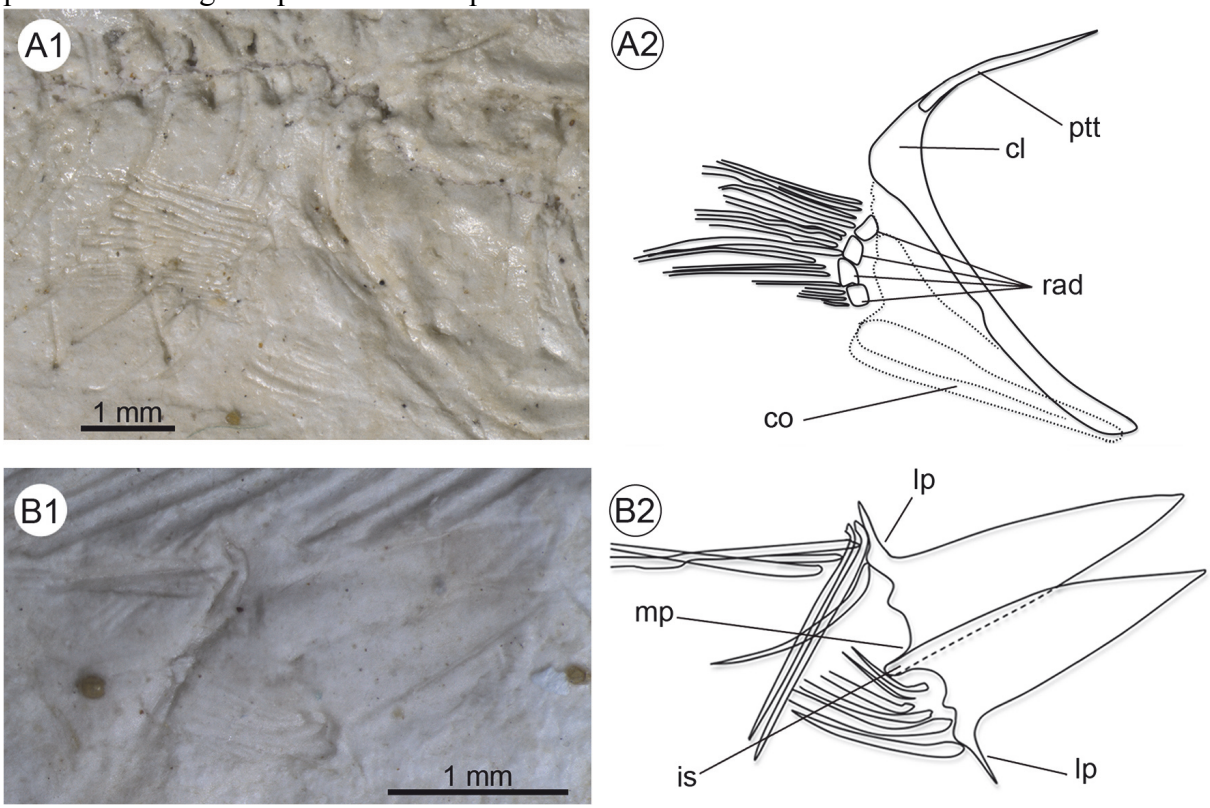


**Figure 6. Details of the pectoral and pelvic girdles and fins seen in †*Kenyaichthys kipkechi* sp. nov., right lateral views**. **A1–A2** Pectoral girdle and fin (paratype 1192´04); **B1–B2** Pelvic girdle and fin (paratype 1218a´05). **Abbreviations**: **cl**, cleithrum; **co**, coracoid; **is**, ischial process; **lp**, lateral process; **mp**, medial process; **ptt**, posttemporal; **rad**, pectoral radials.

**Paired fins.** The rounded pectoral fins are ventrolaterally inserted and of moderate size. The number of rays is 11–16; the tips of the rays do not reach the origin of the pelvic fins (Tables 1, 2 and S3, Figs. 4I and 6A1).

The pelvic fins are small and round, insert beneath vertebrae 8–12 and are positioned closer to the anal fin than to the pectoral fins (Table 1: P–A vs. V–A, and Table 2). The number of rays is 5–7 (Tables 2 and S3, Fig. 6B1–6B2).

Dorsal fin. The relatively small dorsal fin is inserted behind the middle of the standard length (predorsal distance 58.4 ± 2.1, see Table 1). It consists of 13–17 rays, of which the first one or two are clearly discernible as short and unbranched (Tables 2 and S3, Fig. 7). Apart from the last ray, a long proximal radial supports each of the rays, whereas two proximal radials support one of the anteriormost rays. The last ray is not supported by a proximal radial in most cases.

The arrangement of the proximal radials in the dorsal fin is recognizable in 53 specimens of †*K. kipkechi* and shows pronounced polymorphism (see Table S6). Where only one short ray is present, two proximal radials support this ray (seen in 11 specimens) or the first long ray (seen in 12 specimens; Fig. 7A1–7A2).

If two short rays are present, two proximal radials can either support the first short ray (seen in 15 specimens; Fig. 7B1–7B2), or the second short ray (seen in 13 specimens; Fig. 7C1–7C2), or the first long ray (seen in two specimen; Fig. 7D1–7D2). An exception may occur in 1184R´04; it seems to show each ray supported by a single proximal radial, but putative remains of a second proximal radial are recognizable near the first short fin ray. It is therefore coded as 2/1? in Table S6.

**Table 2. Meristic values for †*Kenyaichthys* gen. et sp. nov.** based on the holotype (1209a/b´04) and all other specimens.

| Characters | Holotype | All other specimens |
|---|---|---|
| Dorsal fin rays | 14 | 13–17 |
| Anal fin rays | 18 | 16–22 |
| Pectoral fin rays | 15 | 11–16 |
| Pelvic fin rays | 6 | 5–7 |
| Principal caudal fin rays | ?+8 | 16–22 |
| Procurrent dorsal caudal fin rays | – | 5–15 |
| Procurrent ventral caudal fin rays | 11 | 5–15 |
| Total vertebrae | 33 (15+18) | 29–33 (11–14+17–21) |

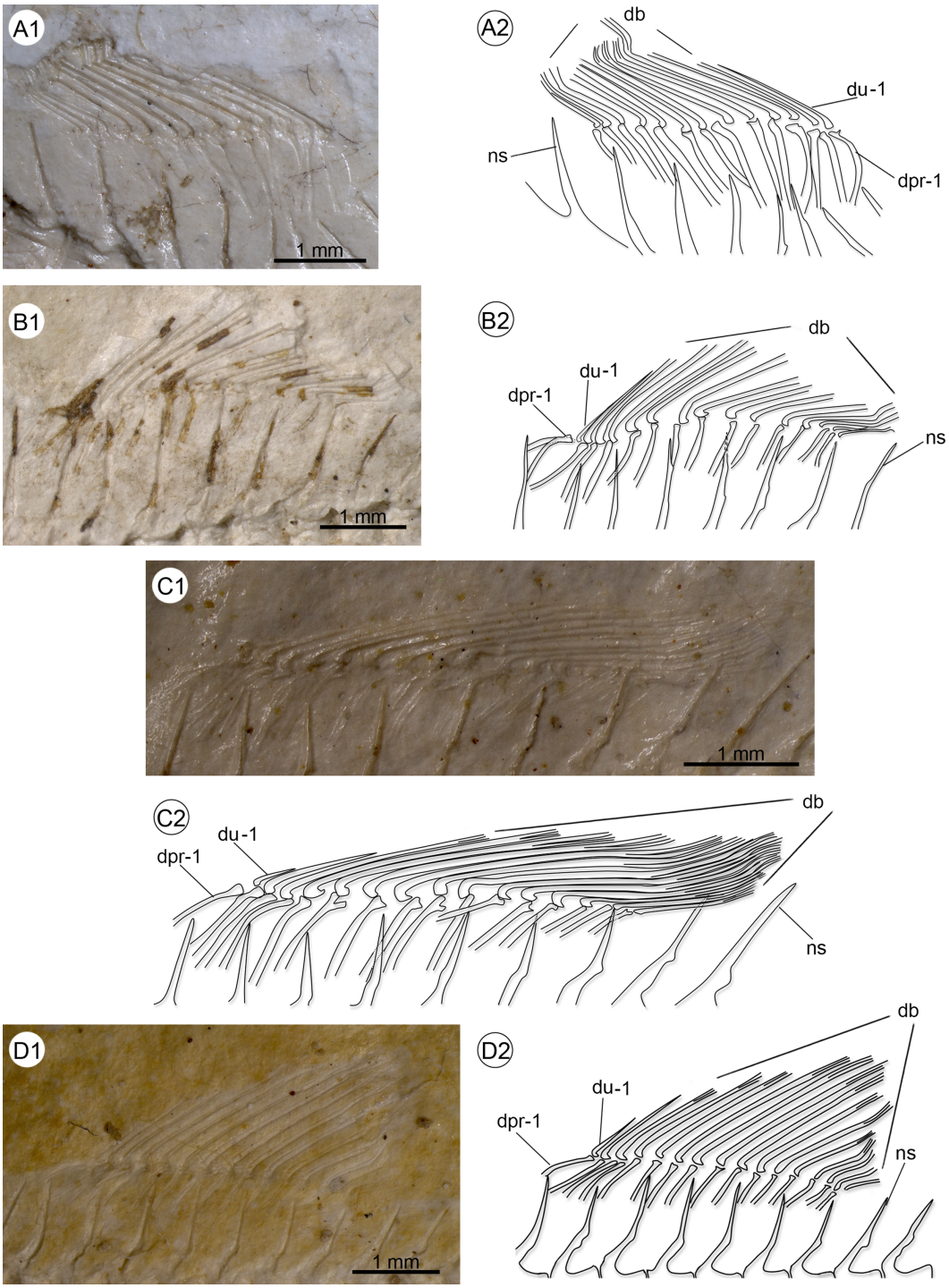


**Figure 7. Polymorphism in the arrangement of the proximal radials of the dorsal fin seen in †*Kenyaichthys* nov. gen**. **A1–A2** †*Kenyaichthys kipkechi* sp. nov. (paratype 1154a´04): dorsal fin with a single short ray and 12 long rays; a single proximal radial supports the first short ray, two proximal radials support the first long ray, and one proximal radial supports all remaining rays, right lateral view; **B1–B2** †*K. kipkechi* sp. nov. (paratype 1152´04): dorsal fin with two short rays and 11 long rays; two proximal radials support the first short ray and one proximal radial supports all other rays with the exception of the last ray, left lateral view; **C1–C2** †*K. kipkechi* sp. nov. (paratype 1206(1)´04): dorsal fin with two short rays and 13 long rays; two proximal radials support the second short ray and one proximal radial supports all other rays, left lateral view; **D1–D2** †*K. kipkechi* sp. nov. (paratype 1168´04): dorsal fin with two short rays and 14 long rays; two proximal radials support the first long ray and one proximal radial supports all other rays with the exception of the last ray, left lateral view. **Abbreviations**: **db**, branched rays of dorsal fin; **dpr-1**, first dorsal proximal radial; **du-1**, first unbranched dorsal fin ray; **ns**, neural spine.

**Anal fin.** The anal fin is slightly larger than the dorsal, and is inserted opposite, slightly behind or in front of the dorsal fin insertion (preanal distance 59.6 ± 2.5, see Table 1). It comprises 16–22 rays, of which only the first up to three are clearly seen to be short and unbranched; each ray (branched or unbranched), generally with the exception of the last, is supported by a single long proximal radial, small medial radials are also recognizable (Tables 2 and S3, Fig. 8).

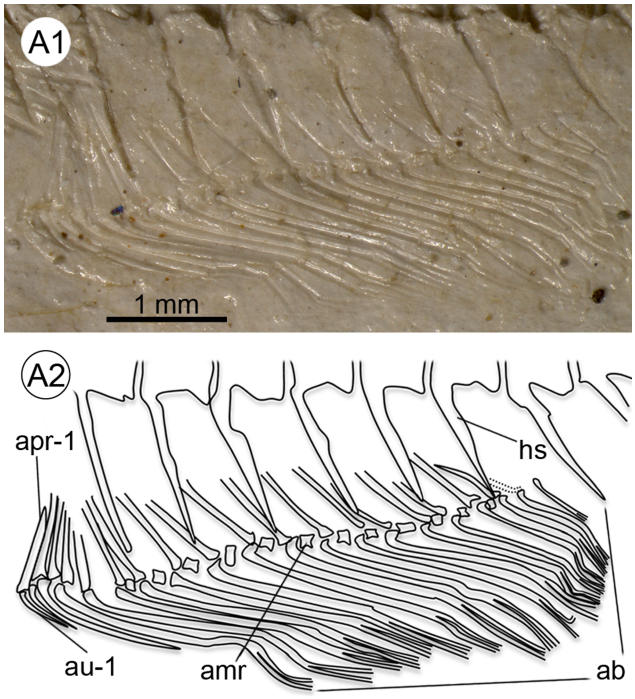


**Figure 8. Details of the anal fin of †*Kenyaichthys kipkechi* sp. nov.** (paratype 1177′04). **A1–A2** Anal fin with three short rays, 17 long rays and 19 proximal radials (last ray not supported by proximal radial), left lateral view. **Abbreviations**: **ab**, branched rays of anal fin; **amr**, anal medial radial; **apr-1**, first anal proximal radial; **au-1**, first unbranched anal fin ray; **hs**, haemal spine.

**Caudal fin.** The caudal fin is small and rounded or truncate in shape (Fig. 3A7). It consistsof 16–21 segmented principal rays (including the branched rays plus the first unbranched ray dorsally and ventrally) and 5–15 short procurrent rays dorsally and ventrally (Table 2 and S3). The segmented and branched principal rays that are supported by the hypural plateform a coherent, uninterrupted array, without any gap in the middle of the plate (Figs. 3A7 and 9). The caudal fin formula for the principal rays is 8– 11/8– 11 (Table S3). The principal rays can extend to the neural and haemal spines of PU2– PU4.

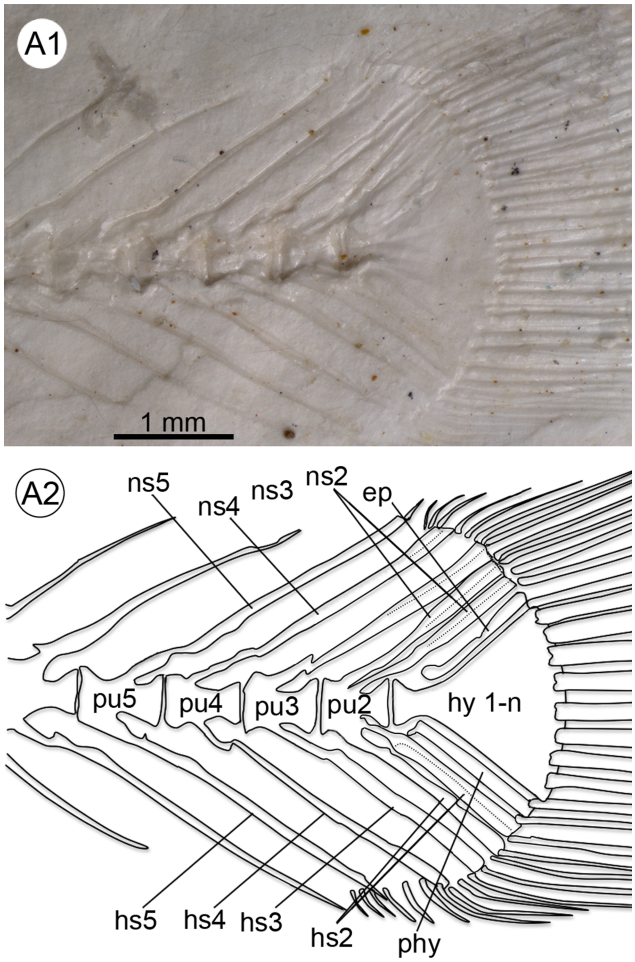


**Figure 9. Details of the caudal skeleton of †*K. kipkechi* sp. nov.** (holotype 1209a′05). **A1–A2** Caudal fin with fused hypural plates, one parhypural, one epural, five preural vertebrae (pu2–5) and duplicated spines of PU2, left lateral view. **Abbreviations**: **ep**, epural; **hs2–5**, haemal spine of preural vertebrae 2–5; **hy 1–n**, hypural plates 1–n; **ns2–5**, neural spine of preural vertebrae 2–5; **phy**, parhypural.

**Caudal skeleton.** The axial skeleton is symmetrical: the terminal centrum is fused to a single triangular hypural plate and joined by a single parhypural and a single epural, each of which provides support for one or two segmented principal rays.

The hypural plate is recognizable in all specimens of †*K. kipkechi* and shows polymorphism in its dimensions (see Tables 1 and S1), with the length ranging from 0.5– 4.9% of SL and the width from 1.1– 5.6% of SL. It is < 1.0% of SL in eight specimens, 1.3– 2.0% in 12 specimens, 2.1– 2.9% in 18 specimens and 3.0– 4.9% of SL in 39 specimens (Fig. 10A–10D).

The extension of the procurrent rays and number of preural vertebrae (PU) is recognizable in 90 specimens; 49 of them possess five preural vertebrae, the remainder have six (see Table S3). The preural vertebrae are characterized by long neural and haemal spines supporting the caudal rays; the neural and haemal spines of the preceding vertebrae are clearly shorter (Fig. 3A7). The proximal portions of the neural and haemal spines of PU2 do not show a constriction.

The uroneural (= stegural) is usually not visible, but in some specimens it is recognizable as a short and tiny structure closely attached to the proximal portion of the upper segment of the hypural plate and terminal centrum (Fig. 2B1–2B2); lateral processes are not recognizable.

Supernumerary neural and/ or haemal spines can be observed in 32 out of 127 specimens in which neural and/ or haemal spines are visible in the caudal region (see Table S3). The additional spine was identified as duplicated if each of the two spines had an individual base (observed in 23 specimens), and as split when both spines shared the same base (nine specimens). Twenty specimens show duplicated haemal and/ or neural spines of PU2. Two specimens show the neural spines of PU5 duplicated, and a single specimen shows duplicated neural and haemal spines on PU3. Five specimens show split neural and/or haemal spines of PU2, and two further specimens display split neural spines of PU3. The remaining two specimens show a split spine of PU2, but it is unclear whether it is the haemal or the neural spine (see Table S3).

The proximal part of the parhypural is recognizable in 78 specimens and is polymorphic, i.e. it may either be reduced and lack contact with the terminal centrum and hypural plate (seen in 64 specimens; Figs. 2B1–2B2 and 9A1–9A2), or articulate with the terminal centrum and at least partially with the hypural plate (seen in two specimens; Fig. 11A1–11A2), or may even be reduced and lack contact with the terminal centrum, but is at least partially fused to the hypural plate (seen in 12 specimens; see Table S6). If the parhypural is reduced, the proximal

part can display a projection that faces away from the terminal centrum and the major part of the bone can be straight (26 specimens) or curved (three specimens); in the remaining 42 specimens the proximal part is continuous with the main axis of the parhypural and the remaining part of the parhypural is straight (33 specimens) or curved (nine specimens). The condition of the parhypural in the remainder five specimens is not recognizable due to insufficient preservation (see Table S6).

The epural is clearly recognizable in 82 specimens and does not make contact with the terminal centrum in any of the specimens, but can be fused to the hypural plate (seen in one specimen). 55 specimens show an epural without an anterodorsal projection; the epural is curved in 35, and straight in 20 of these specimens. The 27 remaining specimens display a straight (19 specimens) or curved (eight specimens) epural with an anterodorsal projection. Additional six specimens have the caudal skeleton preserved, but the orientation of the specimen is unclear (see Table S6).

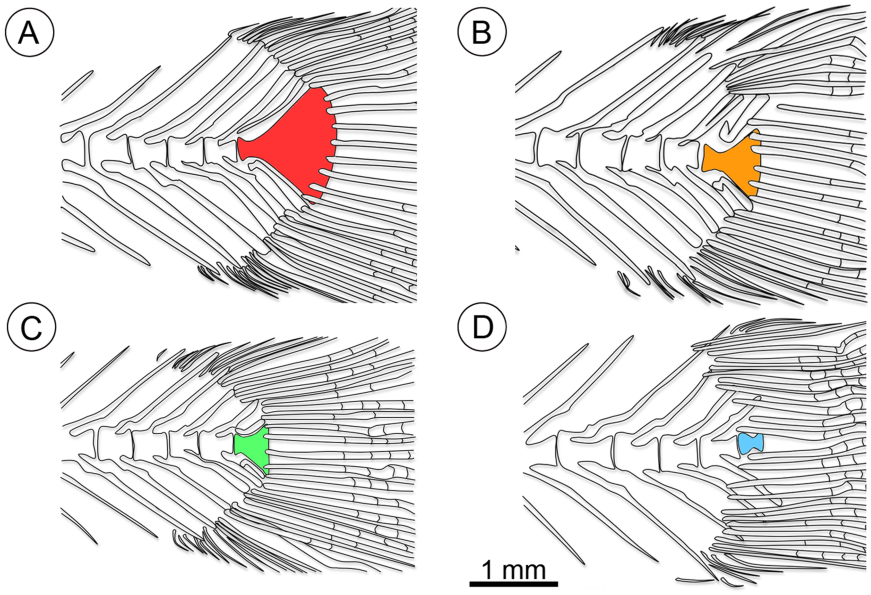


**Figure 10. Reconstructions of the caudal skeletons of four specimens of †*K. kipkechi* sp. nov. showing the polymorphism in the hypural plate dimensions.** **A**, paratype 1237R(1)′04; **B**, paratype 1206(1)′04; **C**, paratype 1168′04; **D**, paratype 1146′04 (mirrored).

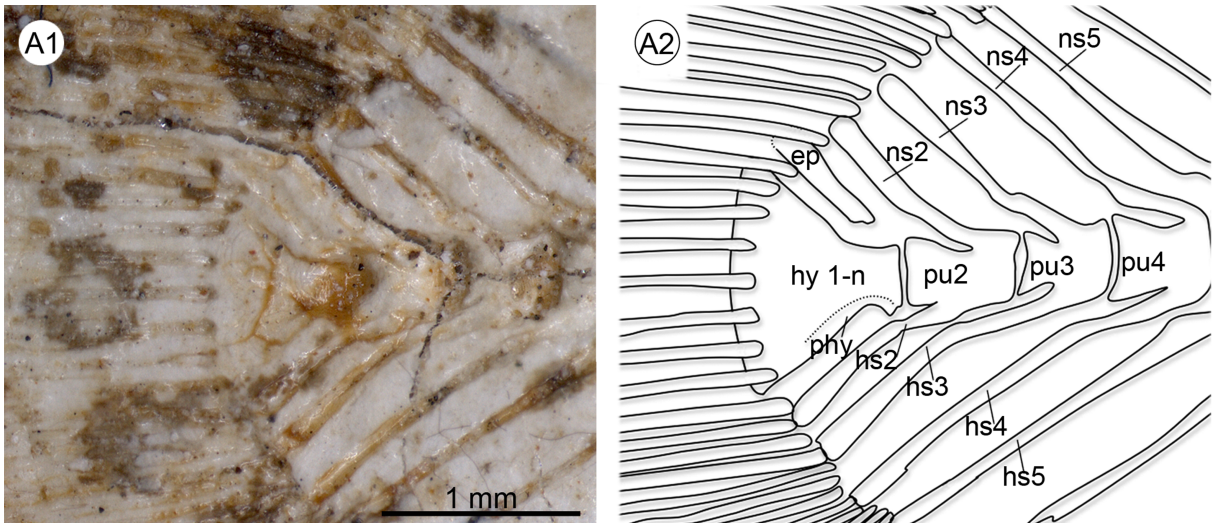


**Figure 11. Details of the caudal skeleton of †*K. kipkechi* sp. nov.** (paratype 1220´04). **A1–2** Caudal fin with overlap between the proximal part of the parhypural, the terminal centrum and the hypural plate, right lateral view. **Abbreviations**: **ep**, epural; **hs2–5**, haemal spine of preural vertebrae 2–5; **hy 1–n**, hypural plates 1–n; **ns2–5**, neural spine of preural vertebrae 2–5; **phy**, parhypural; **pu2–4**, preural vertebrae 2–4.

**Spine ratios.** Both the neural spine PU2/PU4 and PU2/PU5 ratios were > 1.0 in most specimens (Tables 3 and S4). The haemal spine of PU2 is slightly wider than that of PU4 and PU5 in most specimens (ratios > 1.0 and < 2.0), but several specimens revealed also ratios < 1.0 and _ 2.0 (Tables 3 and S4). Furthermore, both the neural and haemal spine PU3/PU5 ratios were > 1.0 in most specimens (Tables 3 and S4).

**Table 3. Spine ratios (means and ranges) of †*Kenyaichthys* gen. et sp. nov. and the recent cyprinodontiform species used for comparison.**

| Species | NS2/NS4 | NS2/NS5 | HS2/HS4 | HS2/HS5 | NS3/NS5 | HS3/HS5 |
|---|---|---|---|---|---|---|
| †*Kenyaichthys* gen. et sp. nov. (all specimens) | 1.6±0.5 / 1.1–4.3 (75) w | 1.8±0.5 / 1.1–3.1 (66) w | 2.4±0.5 / 2.0–3.7 (13) dw | 2.3±0.4 / 2.0–3.7 (19) dw | 1.8±0.5 / 1.1–3.3 (70) w | 1.6±0.5 / 1.1–3.0 (70) w |
| | | | 1.4±0.2 / 1.1–1.9 (48) sw | 1.5±0.2 / 1.2–1.8 (47) sw | | |
| | 0.8±0.2 / 0.5–1.0 (7) ne | 0.9±0.2 / 0.6–1.0 (13) ne | 0.9±0.1 / 0.6–1.0 (15) ne | 0.8±0.1 / 0.6–1.0 (8) ne | 0.9±0.2 / 0.3–1.0 (15) ne | 0.8±0.2 / 0.5–1.0 (14) ne |
| *Aphanius sophiae* | 3.2±1.3 / 1.6–5.4 (19) w | 4.3±1.3 / 2.6–8.0 (19) w | 3.3±0.8 / 2.0–4.4 (17) dw | 4.3±1.2 / 2.8–7.2 (19) dw | 4.0±1.3 / 2.3–6.5 (19) w | 3.9±1.3 / 2.2–6.6 (19) w |
| | | | 1.6±0.3 / 1.5–1.8 (2) sw | – | | |
| | – | – | – | – | – | – |
| *Aphanius farsicus* | 4.1±2.0 / 2.3–7.0 (6) w | 5.0±1.4 / 3.5–7.5 (6) w | 36±2.0 / 2.0–6.2 (5) dw | 5.4±1.6 / 3.5–7.8 (6) dw | 4.3±1.5 / 1.5–5.8 (6) w | 4.8±1.7 / 2.7–7.3 (6) w |
| | | | 1.9 (1) sw | – | | |
| | – | – | – | – | – | – |
| *Aphanius arakensis* | 4.4±3.9 / 2.1–12.0 (6) w | 5.4±1.9 / 2.8–7.7 (5) w | 4.6±3.4 / 2.1–10.5 (5) dw | 6.0±2.2 / 4.0–10.0 (6) dw | 5.4±1.9 / 3.0–7.8 (5) w | 5.5±1.5 / 3.5–7.2 (6) w |
| | | | 1.7 (1) sw | – | | |
| | – | – | – | – | – | – |
| *Aphanius mesopotamicus* | 3.1±0.7 / 2.3–3.5 (3) w | 3.5±1.5 / 2.1–5.7 (4) w | 2.8±0.2 / 2.6–5.3 (3) dw | .7±1.2 / 2.7–5.3 (4) dw | 3.1±0.7 /2.4–4.0 (4) w | 3.7±1.5 / 2.3–5.7 (4) w |
| | | | – | – | | |
| | – | – | 1.0 (1) ne | – | – | – |
| *Pachypanchax playfairii* | 3.2±1.8 / 1.9–4.4 (2) w | 5.0±1.8 / 3.8–6.3 (2) w | 2.3 (1) dw | 3.2±0.6 / 2.8–3.6 (2) dw | 3.2±0.04 3.1–3.2 (2) w | 2.8±0.8 2.3–3.4 (2) w |
| | | | 1.6 (1) sw | – | | |
| | – | – | – | – | – | – |
| Nothobranchiidae | 2.6±0.6 / 1.8–3.7 (10) w | 2.9±1.1 / 1.4–5.4 (9) w | 2.9±0.7 / 2.0–4.0 (7) dw | 3.4±1.4 / 2.0–5.3 (6) dw | 1.9±0.8 / 1.2–3.6 (7) w | 2.2±1.1 / 1.2–4.5 (9) w |
| | | | 1.5±0.1 1.4–1.5 (3) sw | 1.5±0.2 1.3–1.8 (3) sw | | |
| | – | – | – | 1.0 (1) ne | 0.9±0.1 / 0.8–1.0 (2) ne | 1.0 (1) ne |

(), number of specimens; dw, distinctively wider; HS, haemal spine of preural centrum; ne, narrower or equal; NS, neural spine of preural centrum; w, wider; sw, slightly wider. Blank cells indicate unassigned character state.

**Scales.** Cycloid scales (Fig. 12A–12B) are visible on different parts of the body in 98 specimens, of which 22 display scales on the whole body from the preoperculum to the hypural plate. Scales are generally absent from the caudal fin base, with the exception of four specimens that show one to four scales here (1153´04, 1 scale; 1175´04, 3– 4 scales; 1206(1)/1211´04, 1 scale; 1228(1)/1237R(1)´04, 1– 2 scales) (Fig. 4B). The rostral field is only recognizable in the scales on the operculum and the pectoral girdle, which show 7 to 13 radii (Fig. 12A–12B). The squamation pattern on the head that is indicative for the Rivulidae (Costa, 1998a) cannot be identified. Most scales on the body and some scales on the head and hypural plate show an abnormal shape of the central portion, i.e. the scale focus is large and irregular (for scale terminology see (Gholami et al., 2013)) (Fig. 12B). The estimated number of scales in the lateral series is 37– 40 (based on 1237R (1)´04). Mean dimensions of eight key scales (from the third or fourth rows below the dorsal fin) from four different specimens (1171R´04, 1199b´04, 1223R´04, 1237R(1)´04; two scales each) are: 0.44 ± 0.05 mm length (range 0.36– 0.52 mm) and 0.43 ± 0.07 mm width (range 0.36– 0.53 mm) (see Table S5).

**Granulation structures.** 7% (n = 11) of the specimens show a regularly distributed granulation-like structure between the spines and rays of all fins, around the entire vertebral column, on individual vertebrae, and sometimes also on the head (Fig. 12C). Most likely these structures can be interpreted as corrosion of bones in the course of the fossilisation process.

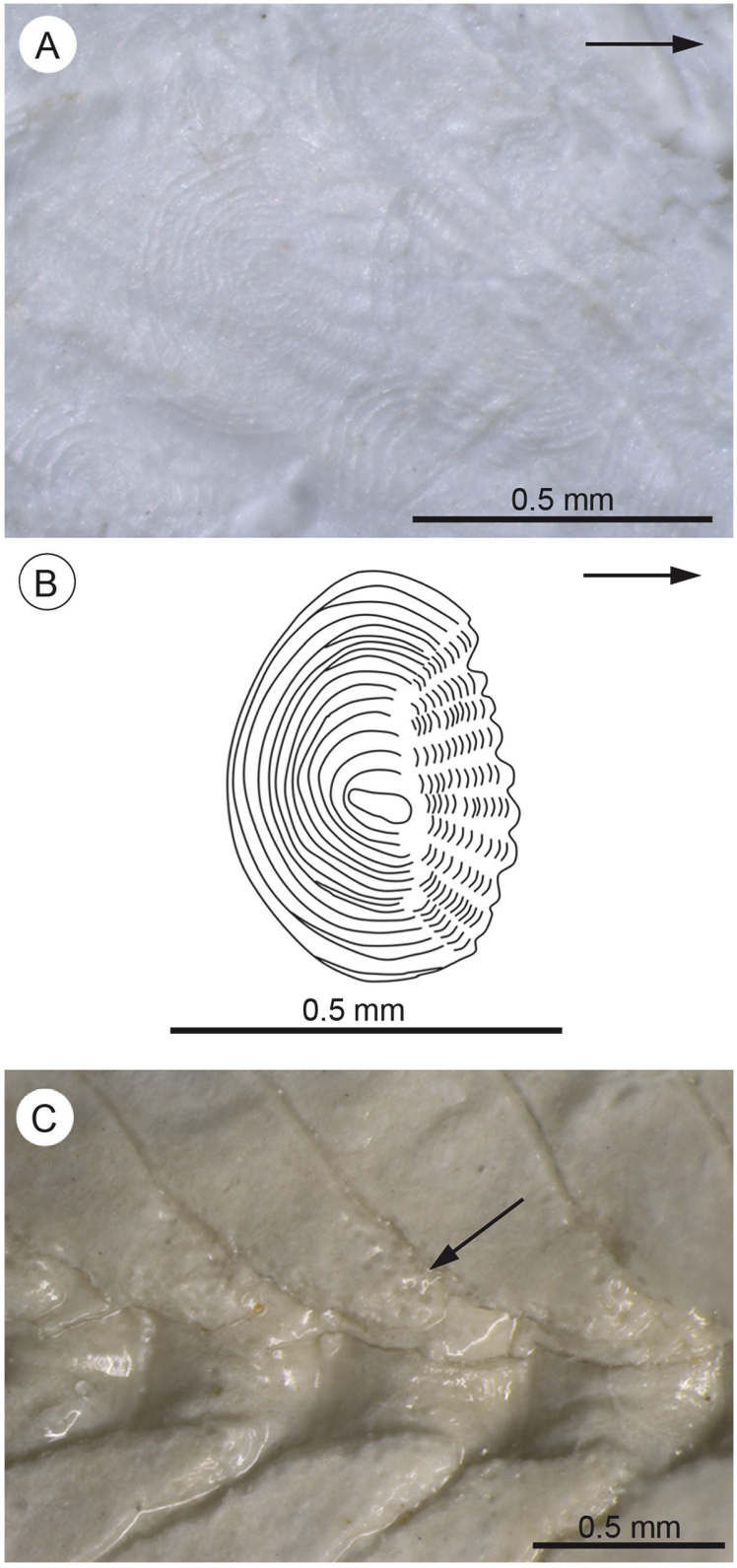


**Figure 12. Details of squamation and granulation structures seen in †*Kenyaichthys* nov. gen**. **A** Cycloid scales on the pectoral girdle of †*Kenyaichthys* cf. *kipkechi* (1223R´04), right lateral view (arrow points anteriorly); **B** Reconstruction of a cycloid scale on the operculum (based on paratype of †*K. kipkechi* sp. nov., specimen 1237R(1)´04), mirrored for better comparison (arrow points anteriorly); **C** Granulation (arrow) on neural spine of caudal vertebra no. 10 (based on †*K. kipkechi* sp. nov., paratype 1147´04), right lateral view.

### 2.3.2. Analysis of extant material

The fossil specimens show variation in the length and width of the hypural plate, in the numbers of preural vertebrae, and also in the width of the haemal and neural spines of the preural vertebrae. In order to understand the taxonomic meaning of this variation, we therefore asked whether these characters show a similar tendency to vary in extant killifish.

**Hypural plate dimensions.** We used four species of *Aphanius* (see comparative material) that all belong to the same young (Holocene) evolutionary lineage based on molecular data (Esmaeli et al., 2014) and therefore represent an excellent model to compare intra- and interspecific variation in closely related species. In all, ten females (8 c&s and 2 xr) and nine males (all c&s) of *A. sophiae*, three females and three males (all xr) of *A. farsicus*, three females (all xr) and three males (2 c&s and 1 xr) of *A. arakensis*, and two females and two males (all c&s) of *A. mesopotamicus* were analysed with regard to the hypural plate dimensions in the two sexes (Tables 4 and S7, mean values and ranges are given in % of SL). The measurements reveal that the hypural plate has a large size range within these four species. However, in *A. sophiae* and *A. arakensis* the hypural plate length is significantly different between males and females (T-Test, $p<0.05$, see Table 4). No unambiguous signals were obtained for such sex dimorphism in *A. farsicus* and *A. mesopotamicus*.

**Table 4. Hypural plate dimensions of the four species of *Aphanius* used for comparison.**

| Species | Sex | n | lH | | wH | |
|---|---|---|---|---|---|---|
| | | | mean | range | mean | range |
| *Aphanius sophiae* | M | 9 (c&s) | 5.83% ± 0.36* | 5.15–6.31% | 8.45% ± 0.48 | 7.71–9.27% |
| | W | 10 (8 c&s, 2xr) | 5.31% ± 0.64* | 4.22–6.49% | 8.03% ± 0.77 | 6.99–9.73% |
| *Aphanius farsicus* | M | 3 (xr) | 5.54 ± 0.48 | 4.99–5.87% | 8.12% ± 0.72 | 7.62–8.95% |
| | W | 3 (xr) | 5.43 ± 0.21 | 5.26–5.66% | 8.07% ± 0.89 | 7.41–9.08% |
| *Aphanius arakensis* | M | 3 (2 c&s, 1 xr) | 5.85% ± 0.22* | 5.60–6.02% | 7.79% ± 0.79 | 7.31–8.71% |
| | W | 3 (xr) | 5.13% ± 0.30* | 4.85–5.45% | 7.50% ± 0.77 | 6.92–8.38% |
| *Aphanius mesopotamicus* | M | 2 (c&s) | 4.93% ± 0.04 | 4.90–4.95% | 7.19% ± 0.66 | 6.72–7.65% |
| | w | 2 (c&s) | 4.47% ± 0.16 | 4.36–4.58% | 7.05% ± 0.09 | 6.98–7.11% |

Significant differences between sexes are indicated with * (T-Test, $p<0.05$).
n, number of specimens; c&s, cleared and stained; lH, length of hypural plate; wH, width of hypural plate; xr, x-ray.

**Number of preural vertebrae.** The degree of within-species variation in preural vertebrae number was examined based on two specimens of the Aplocheilidae (*Pachypanchax playfairii*; two c&s), a total of 10 specimens of the Nothobranchiidae (*Aphyosemion castaneum*, *Epiplatys sexfasciatus*, *Foerschichthys flavipinnis*, *Fundulopanchax sjoestedti*, *Nothobranchius orthonotus*; two c&s each), and a total of 29 specimens of the Cyprinodontidae (*Aphanius sophiae*, 13 seven c&s specimens out of the specimens used above with sufficient preservation of the caudal fin rays; *A. farsicus*, six xr; *A. arakensis*, four xr, two c&s; *A. mesopotamicus*, four c&s). We found intraspecific variation of the preural vertebrae number in *N. orthonotus* (four and five preural vertebrae, see Fig. 13) and in the four examined species of A*phanius* (three and four preural vertebrae). The remaining specimens consistently displayed four preural vertebrae (Table S7). It is therefore clear that intraspecific variation of preural vertebrae number, as observed in †*Kenyaichthys*, is not exceptional as it is also present in extant species.

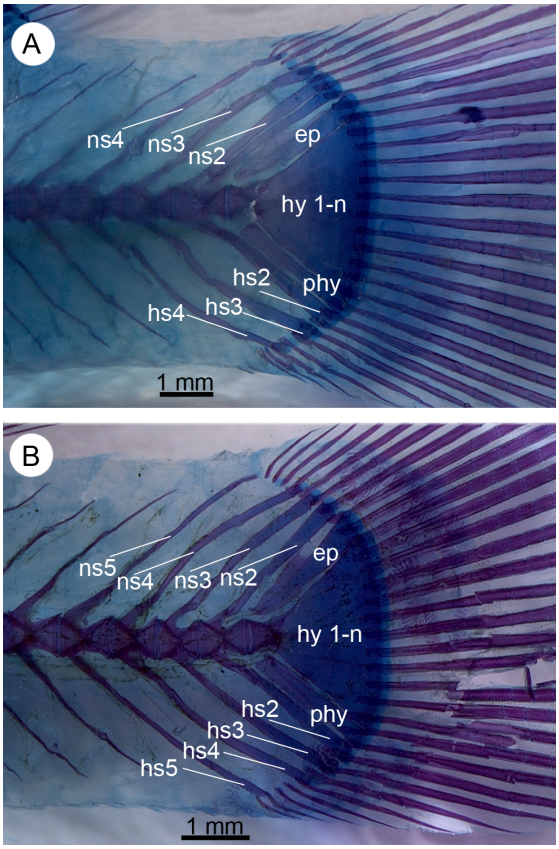


**Figure 13. Intraspecific variation in the number of preural vertebrae in *Nothobranchius orthonotus*** (MRAC A4-039-P-0133-0134). **A** Four preural vertebrae; **B** five preural vertebrae © Royal Museum for Central Africa Tervuren (Belgium). **Abbreviations**: **ep**, epural; **hs2–5**, haemal spine of preural vertebrae 2–5; **hy 1-n**, hypural plates 1–n; **ns2–5**, neural spine of preural vertebrae 2–5; **phy**, parhypural.

**Ratios of neural and haemal spines of preural vertebrae.** The ratios of the widths of haemal and neural spines of PU2/PU4, PU2/PU5 and PU3/PU5 were considered to be important at multiple taxonomic levels in previous studies (see Discussion). They include:

i. a synapomorphy for Cyprinodontiformes vs. Adrianichthyidae (Beloniformes) (neural spine of PU2 wider than neural spines of PU4 and PU5 vs. about equal);

ii. a synapomorphy for Cyprinodontoidei vs. Aplocheiloidei (neural and haemal spines of PU3 wider than spines of PU5 vs. about equal);

iii. a synapomorphy for Nothobranchiidae/Rivulidae vs. all other cyprinodontiform families (haemal spine of PU2 slightly wider than haemal spines of PU4 and PU5 vs. distinctively wider).

In the case of (iii), however, it is not clear from previous studies how “slightly wider” and “distinctively wider” should be defined. We consider here ratios of >1.0 and <2.0 as slightly wider and ratios of 2.0 as distinctively wider.

We used the comparative material described above to verify the phylogenetic significance of these characters. Ratios between spines were calculated based on the maximal width of the respective spine (see Figs. 2B and S1).

i. The neural spine of PU2 was wider than the neural spines of PU4 and PU5 in almost all specimens studied (Tables 3 and S8), as expected for a cyprinodontiform species (see Table 5). The single exception is specimen ZM-CBSUZG 363 of *Aphanius mesopotamicus*, which reveals the neural spine of PU2 as wide as the neural spine of PU4.

ii. The neural and haemal spines of PU3 were wider than those of PU5 in the cyprinodontoid specimens (Tables 3 and S8). However, PU3 neural and haemal spines were also wider than PU5 spines in nine and 11 of the aplocheiloid specimens, respectively (Tables 3 and S8), rather than being about equal as expected for the Aplocheiloidei from previous work (see Table 6). The mean values of the aplocheiloid specimens are significantly smaller than that of the cyprinodontoid specimens (T-Test, $p < 0.0001$ for neural and haemal spine ratios), however, the ranges of PU3/PU5 ratios overlap between the two groups (Tables 3 and S8).

iii. In seven of the ten studied extant nothobranchiid specimens, the haemal spine of PU2 is distinctively wider (ratio 2.0) than those of PU4. Moreover, six of the ten specimens show a ratio of _ 2.0 for PU2/PU5. However, only the character state “ slightly wider” (1.0< ratio < 2.0) is expected for Nothobranchiidae and Rivulidae from previous work (see above and Table 7).

Moreover, ranges for PU2/PU4 and PU2/PU5 ratios display overlap between all studied species (Tables 3 and S8), and the mean values of the nothobranchiid specimens are not significantly smaller than seen in the aplocheilid species *P. playfairii* (T-Test, $p > 0.05$). On the other hand, the PU2/PU5 mean value for the nothobranchiid species is significantly smaller than in the studied cyprinodontoid species (T-Test, $p < 0.01$), as expected from literature data.

Apart from those few specimens that show the neural spine smaller to equal to those of PU4 and/or PU5, all specimens of †*Kenyaichthys* exhibit a neural spine on PU2 that is wider than those of PU4 and PU5 (see above), like the studied extant cyprinodontiform species, with the exception of one specimen of *Aphanius mesopotamicus* (see Table 3). Furthermore, †*Kenyaichthys* displays mean values and ranges of PU3/PU5 neural and haemal spine ratios that are comparatively close to the mean values of the studied aplocheiloid specimens (see Table 3). In addition, the haemal spine PU2/PU4 mean value of †*Kenyaichthys* is closer to the respective value of the studied aplocheilid specimens, whereas the haemal spine PU2/PU5 mean value of †*Kenyaichthys* is closer to the respective value of the studied nothobranchiid specimens (see Table 3).

### 2.3.3. Phylogenetic reconstruction

To elucidate the systematic position of †*Kenyaichthys*, a phylogenetic analysis based on 72 morphological characters was conducted (according to Costa (1998a, 2004 and 2012)); see Tables S9 and S10 and S1 NEXUS File) (Figs. 14 and 15). The character " spines of PU3 wider vs. equal compared to the spines of PU5" (Costa, 2012) was not used because our data obtained from the extant specimens indicated that the PU3/PU5 ratios of neural and haemal spines cannot be reliably used to separate cyprinodontoid from aplocheiloid species. The character " haemal spine of PU2 slightly vs. distinctively wider compared to PU4 and PU5" (Costa, 2012) was also omitted because our data showed that the PU2/PU4 and PU2/PU5 ratios of the haemal spines overlap between the Aplocheilidae and Nothobranchiidae (see above). Furthermore, we discarded the character " mouth position superior vs. terminal" because, based on the studies of Costa (1998a, 2004, 2013), it has not been unambiguously determined whether the superior mouth position is an apomorphic or plesiomorphic trait. Furthermore, the character " 12 to 16 and 20 to 25 radii vs. four to six radii on the anterior

abdominal scales" (Costa, 1998a) could not be checked in the fossils because †*Kenyaichthys* only sparsely revealed radii on its scales, and such scales were exclusively found near the shoulder girdle and the operculum. The condition of this character in †*Kenyaichthys* was therefore coded as "?" in the character matrix (see also Tables 5 and 6). It should be noted that the presence of a pelvic girdle lateral process could not yet be used in phylogenetic analyses because this character remains to be explored for most extant groups.

The outcome of the phylogenetic analysis clearly places †*Kenyaichthys* within the Aplocheiloidei (Figs. 14 and 15). It emerges as sister to the Rivulidae, while †*Kenyaichthys* together with Rivulidae are sister to the Aplocheilidae and Nothobranchiidae, which appear as unresolved polytomy. The Cyprinodontoidei display the same topology as in previous studies.

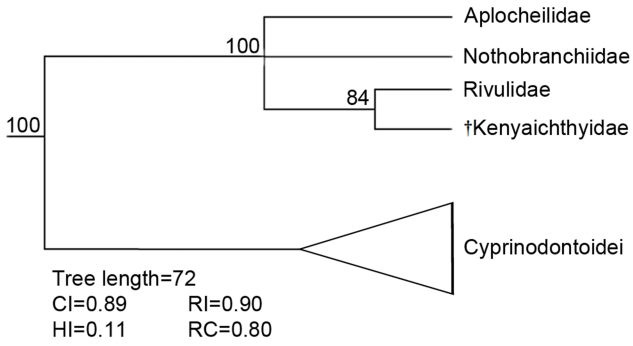


**Figure 14. 50% majority-rule consensus tree for the Cyprinodontiformes and †*Kenyaichthys* gen. et sp. nov.** (red arrow) based on 72 morphological characters as used in the studies of Costa (1998a, b, 2004, 2009, 2012) created using PAUP (Swofford, 2003). Numbers above nodes refer to bootstrap values (based on 1000 replicates). Abbreviations: CI, consistency index; HI, homoplasy index; RI, retention index; RC, rescaled consistency index.

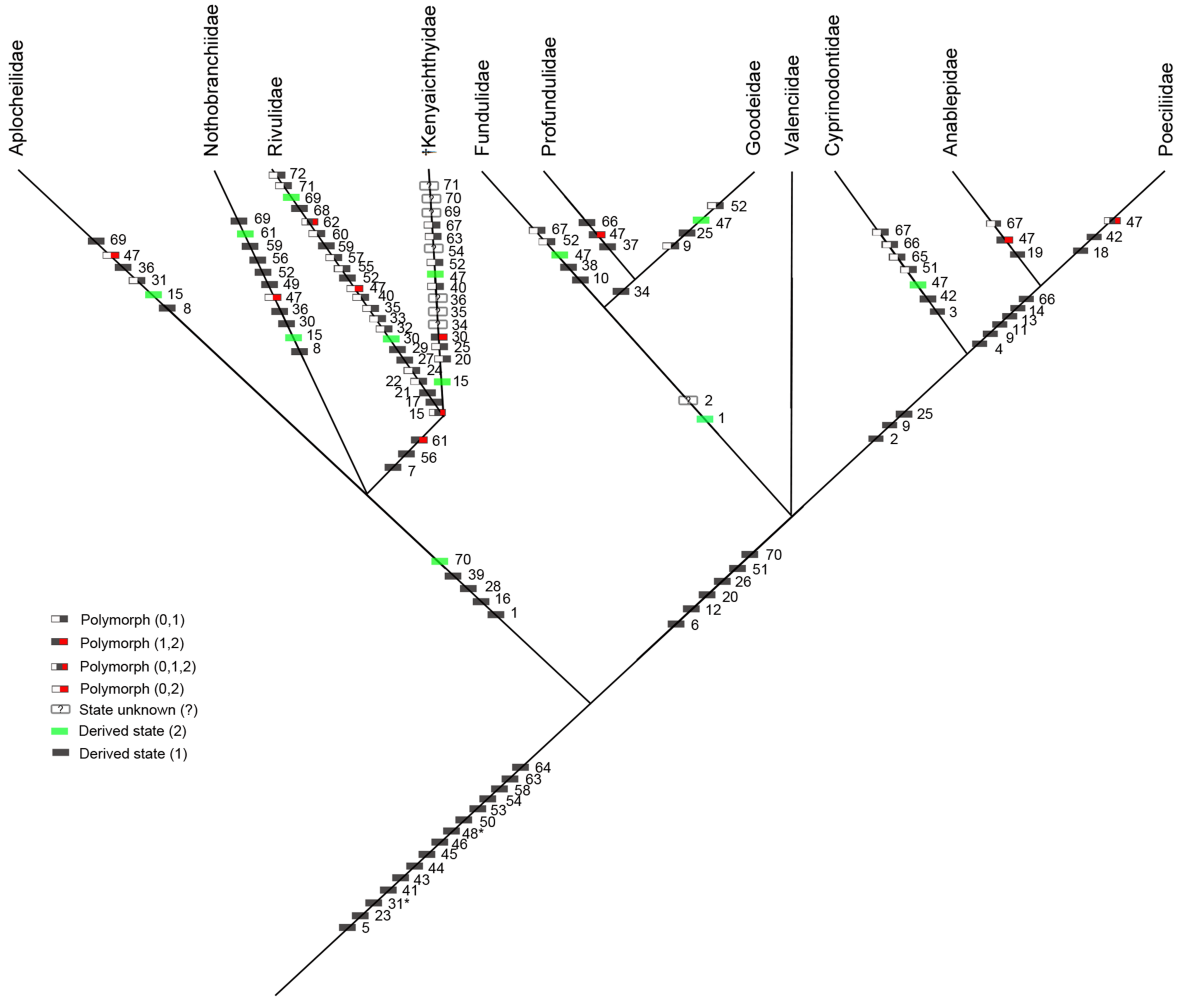


**Figure 15. 50% majority-rule consensus tree for the Cyprinodontiformes and †*Kenyaichthys* gen. et sp. nov. with all the 72 morphological characters mapped.** * Indicates character reversals: *character: 31 synapomorphy for all Cyprinodontiformes, but reversal in some Poeciliidae and Aplocheilus; *character 48: synapomorphy for all Cyprinodontiformes with reversal in some Nothobranchiidae and Aplocheilidae.

## 2.4. Discussion

### 2.4.1. Relationship of †*Kenyaichthys* to Cyprinodontiformes

The foregoing investigation of our †*Kenyaichthys* material reveals that it exhibits 12 of the 19 synapomorphies that are diagnostic for the Cyprinodontiformes (Table 5), among them the well-developed neural spine of PU2. While most of the studied fossil specimens of †*Kenyaichthys* possess a neural spine of PU2 that is wider than the neural spines of PU4 and PU5, seven specimens displayed the neural spine of PU2 not wider than that of PU4, and 13 specimens showed the neural spine of PU2 not wider than the neural spine of PU5 (see Table 3). This condition has only been described for the Adrianichthyidae (Order Beloniformes) (Costa, 2012), which is sister to the Cyprinodontiformes (Nelson, 2006; Hertwig, 2008; Betancour-R. et al., 2013).

However, our data reveal that exceptions may occur as observed in *Aphanius mesopotamicus* for the neural spine PU2/PU4 ratio. In Atheriniformes and other Beloniformes the neural spine of PU2 is not fully developed (Costa, 2012). Beloniformes are characterized by (among other traits) a " lower lobe of the caudal fin with more principal fin rays than [the] upper lobe" (Wiley and Johnson (2010) page 156). This is definitely not the case in six specimens, (1177´04, 1194´04, 1206(1)/1211´04, 1218´04, 1227´04, and 1228(1)/1237R(1) ´04) and cannot be ascertained in the remaining specimens. We therefore confidently assign the genus †*Kenyaichthys* to the Cyprinodontiformes.

**Table 5. Summary of the osteological synapomorphies for the Cyprinodontiformes and comparisons with †*Kenyaichthys* gen. et sp. nov.** compiled from Rosen (1964), Parenti (1981), Rosen and Parenti (1981), Costa (1998a), Costa (2004), and Costa, (2012).

| Synapomorphy (author and character number) | Cypr | †*Ken* |
|---|---|---|
| Distinct expansion of the alveolar arm of premaxilla (Parenti (1981); Costa (1998a), char. 13) | + | + |
| Dorsal edge of mesopterygoid reduced (Costa (1998a), char. 32) | + | + |
| Urohyal deep (Costa (1998a), char. 37) | + | n.a. |
| Ventral process of the lateral portion of second epibranchial absent (Costa (1998a), char. 55) | + | n.a. |
| Mesethmoid region slightly anterior to lateral ethmoid (Costa (1998a), char. 70) | + | n.a. |
| Ventrolateral pectoral fin insertion (Parenti (1981); Costa (1998a), char. 74) | + | + |
| First postcleithrum scale-like (Parenti (1981); Costa (1998a), char. 78) | + | n.a. |
| Anteromedial process of pelvic girdle absent (Costa (1998a), char. 84) | + | + |
| Caudal fin skeleton symmetrical (Rosen (1964); Parenti (1981); Costa (1998a), char. 86; Costa, (2004), char. 37) | + | + |
| Caudal fin truncate or rounded (Rosen (1964); Parenti (1981); Rosen and Parenti (1981) Costa (1998a), char. 87; ) | + | + |
| Caudal fin rays continuously arranged (Costa (2012), char. 3) | + | + |
| Complete ankylosis of upper hypurals and terminal centrum (Costa (2012), char. 7) | + | + |
| Stegural minute (Costa (2012), char. 5) | + | + |
| One single epural (Rosen (1964); Parenti (1981); Costa (2012), char. 1) | + | + |
| Blade-like epural (Rosen (1964); Costa (2012), char. 2) | + | ? |
| First pleural rib on second vertebra (Rosen (1964); Parenti (1981); Rosen and Parenti (1981); Costa (1998a), char. 95) | + | + |
| Preural vertebra 2, well-developed neural spine with distal tip acting in support of caudal fin rays (Costa (2012), char. 4) | + | + |
| Preural vertebra 2, neural spine wider than neural spines of preural vertebrae 4 and 5 (Costa (2012), char. 6) | + | P |
| 12–16 or 20–25 radii on anterior abdominal scales (Costa (1998a), char. 105) | + | ? |

+, present; P, polymorphic; n.a., not applicable; ?, uncertain; Cypr =Cyprinodontiformes; †*Ken*=†*Kenyaichthys*.

### 2.4.2. †*Kenyaichthys*–A member of Aplocheiloidei or Cyprinodontoidei?

Costa (1998a, 2004, 2009, 2012) provided a series of synapomorphies for the further classification of killifishes (Tables 6–7) of which several were based on osteological characters and are thus in principle applicable to fossils. Seventeen osteological synapomorphies define the Aplocheiloidei and 13 osteological synapomorphies are diagnostic for the Cyprinodontoidei (Table 6), but 12 and six of them, respectively, refer to delicate structures that are not preserved in the fossils studied here (Table 6). We have found that †*Kenyaichthys* displays four synapomorphies of the Aplocheiloidei, namely the presence of a short dorsal process on the maxilla that is anteriorly directed and probably not parallel to the ventral process (Fig. 3A4–3A5), a reduced coronoid process on the anguloarticular (Fig. 3A4–3A5), a flattened neurocranium (Figs. 3A2, 4B, 4C1 and 4D1), and a short medial process of the pelvic girdle (Fig. 6B1–6B2). Of the 13 osteological synapomorphies that

define the Cyprinodontoidei, one concerns the width of the spines of PU3 relative to those of PU5. According to Costa (2012), in Cyprinodontoidei the spines of PU3 are wider than those on PU5 (apomorphic condition), whereas the Aplocheiloidei have narrow neural and haemal spines of PU3 that are no wider than their counterparts on PU5 (plesiomorphic condition). However, our data derived from the extant specimens clearly indicate that (i) species assigned to the Aplocheiloidei do not consistently exhibit narrow neural and haemal spines of PU3, and that (ii) the PU3/PU5 ratios can overlap with those of the Cyprinodontoidei (see above). In †*Kenyaichthys* the spines of PU3 are wider than those of PU5 (with the exception of 16 and 14 specimens, for neural and haemal spines respectively), but the range of the PU3/PU5 ratios is comparatively close to that of the studied aplocheiloid specimens (see above and Table 3). This might be an additional hint that the fossil material belongs to the Aplocheiloidei, as deduced from the other synapomorphies mentioned above.

Moreover, †*Kenyaichthys* displays two characters that appear to be intermediate between the extant Cyprinodontoidei and Aplocheiloidei, one at subordinal level, the other at family level. One is the curvature of the autopalatinum head, which is not straight as seen in the Aplocheiloidei (= plesiomorphic state), but also not as sharply bent as is typical for the Cyprinodontoidei (= apomorphic state; see Costa (1998a) page 542 and Fig. 4F). The second feature is the shape of the posterior margin of the quadratum, which does not show the strongly concave form seen in the two cyprinodontoid families Profundulidae and Goodeidae (= apomorphic state; see Costa (1998a) page 544 and Fig. 4E–4F), but is not as rounded as in the aplocheiloid and remaining cyprinodontoid families. The presence of such " intermediate" characters could indicate that †*Kenyaichthys* is in a "premature" evolutionary state (see below).

Another interesting character of †*Kenyaichthys* is the presence of five or six preural vertebrae, whereas Aplocheiloidei and Cyprinodontoidei usually possess four or five preural vertebrae.

Six preural vertebrae have only been reported for three distantly related cyprinodontoids, i.e. the anablepid *Anableps*, the cyprinodontid *Orestias* and the fundulid *Fundulus* (Costa, 2012). Costa (2012) argued that the increase in the number of preural vertebrae has evolved independently in these genera. It is therefore possible that this trait is an independent acquisition in †*Kenyaichthys* as well and has no taxonomic meaning.

In summary, †*Kenyaichthys* displays a unique combination of characters, four of which are apomorphic for the Aplocheiloidei, two are intermediate, and one may represent parallel

evolution. Together with the phylogenetic analysis (Figs. 14 and 15), these data support the interpretation of †*Kenyaichthys* as a member of the Aplocheiloidei.

**Table 6. Summary of the osteological synapomorphies for the Cyprinodontoidei and Aplocheiloidei and comparisons with †*Kenyaichthys* gen. et sp. nov.** compiled from Parenti (1981), Costa (1998a, 2004 and 2012).

| Synapomorphy (author and character number) | Cypr | Apl | †*Ken* |
|---|---|---|---|
| Posterior indentation of the alveolar arm of premaxilla (vs. absent) (Parenti (1981); (Costa (1998a), char. 14) | + | 0 | 0 |
| Dentary deep (vs. slender) (Parenti (1981); Costa (1998a), char. 19) | + | 0 | 0 |
| Head of autopalatinum bent anteriorly, displaced laterally relative to the main axis of the bone (vs. continuous with the main longitudinal axis of the bone) (Costa (1998a), char. 27) | + | 0 | I |
| Metapterygoid absent (vs. present) (Parenti (1981); Costa (1998a), char. 34) | + | 0 | 0 |
| Dorsal hypohyal absent (vs. present) (Parenti (1981); Costa (1998a), char. 41) | + | 0 | n.a. |
| First basibranchial absent (vs. present) (Parenti (1981); Costa (1998a), char. 45) | + | 0 | n.a. |
| Ventral process of fourth ceratobranchial expanded medially (vs. short) (Costa (1998a), char. 48) | + | 0 | n.a. |
| Ventral process of lateral portion of second epibranchial absent (vs. present) (Costa (1998a), char. 55) | + | 0 | n.a. |
| Second pharyngiobranchial expanded ventrally (vs. not expanded) (Costa (1998a), char. 58) | + | 0 | n.a. |
| Lacrimal approximately rectangular (vs. approximately triangular) (Costa (1998a), char. 71) | + | 0 | 0 |
| Neuroapophyses on the first vertebra separated (vs. united) (Costa (1998a), char. 96) | + | 0 | n.a. |
| Stegural, ventral portion with lateral spine-like process (vs. no spine-like process) (Costa (2012), char. 10) | + | 0 | 0 |
| Neural and haemal spines of PU3 wider than spines anterior to PU4 vs. about equal (Costa (2012), char. 9) (see Text) | + | 0 | P |
| Dorsal process of maxilla short, anteriorly directed, not parallel to ventral process (vs. long, medially directed or vestigial) (Costa (1998a), char. 1) | 0 | + | + |
| Main axis of the ventral process of maxilla slightly curved, tip directed posteriorly (vs. directed anteriorly) (Costa (1998a), char. 4) | 0 | + | n.a. |
| Coronoid process of anguloarticular reduced (vs. not reduced) (Costa (1998a), char. 23; Costa (2004), char. 7) | 0 | + | + |
| Lateral flange of hyomandibula expanded posterodorsally (vs. short) (Costa (1998a), char. 35) | 0 | + | n.a. |
| Anterior portion of basihyal widened (vs. slender) (Costa (1998a), char. 43) | 0 | + | n.a. |
| A distinct anteromedial process on second hypobranchial directed toward second basibranchial (vs. absent) (Costa (1998a), char. 46) | 0 | + | n.a. |
| A distinct posterior process on fourth epibranchial (vs. absent) (Costa (1998a), char. 57) | 0 | + | n.a. |
| Dentition on second pharyngiobranchial reduced (vs. not reduced) (Costa (1998a), char. 59) | 0 | + | n.a. |
| Vomerine teeth present (vs. absent) (Parenti (1981); Costa (1998a), char. 60) | 0 | + | n.a. |
| Wide process on the anterior portion of lateral ethmoid (vs. narrow or no process) (Costa (1998a), char. 63) | 0 | + | n.a. |
| Neurocranium flattened (vs. not flattened) (Costa (1998a), char. 66; Costa (2004), char. 30) | 0 | + | + |
| Dermosphenotic short (vs. elongate or minute) (Costa (1998a), char. 73) | 0 | + | n.a. |

**Table 6. (Continued)**

| | | | |
|---|---|---|---|
| Medial process of pelvic girdle short (vs. long) (Parenti (1981); Costa (1998a), char. 83) | 0 | + | + |
| Distal radial of anal fin with an expanded posteroventral rim (vs. without ventral extensions) (Costa (1998a), char. 93) | 0 | + | n.a. |
| Supraorbital canals open with neuromasts exposed externally (vs. closed) (Parenti (1981); Costa (1998a), char. 98) | 0 | + | n.a. |
| Anterior naris opening at the tip of a distinctively cylindrical structure (vs. flat, no fleshy structure or situated on prominent fleshy structure) (Costa (1998a), char. 100) | 0 | + | n.a. |
| 20 to 25 radii on anterior abdominal scales (vs. 12 to 16) (Costa (1998a), char. 105) | 0 | + | ? |

+, present; 0, absent; P, polymorphic; n.a., not applicable; I, intermediate; ?, uncertain; Cypr=Cyprinodontoidei; Apl=Aplocheiloidei; †*Ken* =†*Kenyaichthys*.

### 2.4.3. Relationships of †*Kenyaichthys* within the Aplocheiloidei

We have also considered the synapomorphic characters of the extant aplocheiloid families, i.e. Aplocheilidae, Nothobranchiidae and Rivulidae (Table 7). However, the only synapomorphy for the Aplocheilidae sensu strictu (according to Costa (2004)) is a black spot on the dorsal fin of the females, a character that cannot be assessed in a fossil. Therefore we have used here and in Table 7 the synapomorphies that were defined for the clade of the Aplocheilidae sensu Costa (1998a), which comprises the Nothobranchiidae + Aplocheilidae as used in later studies.

1. Apomorphic characters of †*Kenyaichthys* shared with the Aplocheilidae sensu Costa (1998a) are an expanded ventral process of the anguloarticular (Fig. 4A1–4A2) and (probably) a supracleithrum fused to the post-temporal (Fig. 6A1–6A2). On the other hand, †*Kenyaichthys* lacks the medially curved premaxillary ascending process that is also diagnostic for this group (this process is flat in †*Kenyaichthys*, see Fig. 4C).

2. †*Kenyaichthys* does not display the two osteological autapomorphies for the Nothobranchiidae, i.e. bifid epipleural ribs (rod-shaped in †*Kenyaichthys*, Figs. 3A6 and 4D3) and a keel-shaped lateral process on the middle part of the terminal centrum (smooth in †*Kenyaichthys*, Figs. 2B1–2, 9, 10 and 11).

3. †*Kenyaichthys* displays three derived characters of the Nothobranchiidae and Rivulidae, i.e. a probably twisted and reduced lacrimal, a distinctive neural spine on the first vertebra (Fig. 4D1–4D4), and a dorsal fin with one or two short rudimentary rays in front of the first long ray (Fig. 7A1–7D2). A further synapomorphy for the Nothobranchiidae and Rivulidae defined by Costa (2004, 2012) is a narrow haemal spine of PU2, which is only slightly wider than the haemal spines of PU4 and PU5 (vs. distinctively wider in Aplocheilidae and

Cyprinodontoidei). However, the phylogenetic value of this character remains to be explored in more detail, because the studied extant species of the Cyprinodontoidei, Aplocheilidae and Nothobranchiidae show a large degree of overlap in the respective ratios (see above).

4. †*Kenyaichthys* shares one autapomorphy with the Rivulidae, i.e. a premaxilla with a straight posterior border, whereas other characters clearly do not display the apomorphic state of the Rivulidae (Table 7). These comprise the broad coronoid process of the dentary (Fig. 3A4–3A5) vs. a narrow coronoid process in Rivulidae; the ventral tip of the autopalatinum, which is long and extends to the quadratum (Figs. 3A2–3A3 and 4A1–4A2) vs. shortened and not reaching the quadratum in Rivulidae; the robust and approximately L-shaped preoperculum (Fig. 4A1–4A2) vs. thin and C-shaped in Rivulidae; and the lack of reduction in the lateral rim of the frontal (Fig. 4B) vs. reduced in Rivulidae. In addition, it is possible that the branchiostegal and opercular membranes were separated in †*Kenyaichthys* (vs. united in Rivulidae) because †*Kenyaichthys* does not display scales on the branchiostegal rays (Fig. 4A1), whereas continuous squamation on the ventral side of the head would be expected if the two membranes were united (Parenti (1981) page 376).

Clearly, †*Kenyaichthys* possesses a combination of apomorphic characters that is not diagnostic for any of the extant aplocheiloid families. The possession of one or two short dorsal fin rays in front of the first long ray indicates that †*Kenyaichthys* is nearer to the Nothobranchiidae and Rivulidae than to any other extant family, which is supported by the phylogenetic analysis (Figs. 14 and 15). Notably, and in contrast to our expectation, the phylogenetic tree places †*Kenyaichthys* closer to the Rivulidae, which represents a purely Neotropical group, than to the Aplocheilidae or Nothobranchiidae, which are widespread on the African continent (and on Madagascar and in India). This is probably due to the mutual possession of the distal portion of the premaxilla with a straight posterior border (in Rivulidae and †*Kenyaichthys*), the presence of a single mutual synapomorphy with the Aplocheilidae + Nothobranchiidae (= Aplocheilidae sensu Costa (1998a)), and the lack of shared synapomorphies with the Nothobranchiidae or Aplocheilidae alone (see Table 7). We therefore consider the sister relationship of †*Kenyaichthys* and Rivulidae suggested by the phylogenetic analysis to be biased, due to the lack of equally available synapomorphies for the Rivulidae, Nothobranchiidae and Aplocheilidae.

Additional apomorphies for the Aplocheilidae and Nothobranchiidae, found in future work, may well shift the phylogenetic position of †*Kenyaichthys* towards these two families, as

would be expected based on their present biogeography on the African continent, Madagascar and India. On the other hand, the hitherto complete lack of information on the aplocheiloid fossil record may mean that current phylogenetic reconstructions are misleading in some respects. An alternative explanation for the observed tree topology is that the premaxilla character described above is not an apomorphy for the Rivulidae, but was in the past shared with lineages of aplocheiloids that are no longer extant.

**Table 7. Summary of the osteological synapomorphies for the Aplocheilidae (sensu Costa (1998a)), Nothobranchiidae and Rivulidae and comparisons with †*Kenyaichthys* gen. et sp. nov.** compiled from Parenti (1981), Costa (1990, 1998a, b, 2004, 2009 and 2012).

| Synapomorphy (author and character number) | Apl | Noth | Riv | †*Ken* |
|---|---|---|---|---|
| Ventral process of anguloarticular expanded (vs. not expanded) (Costa (1990); Costa (1998a), char. 22) | + | + | 0 | + |
| Supracleithrum and posttemporal coossified (vs. not fused) ((Parenti, 1981); (Costa, 1998a), char. 76; Costa (2004), char. 48) | + | + | 0 | +? |
| Posterior tip of the ascending process of premaxilla curved medially (vs. plan) (Parenti (1981); Costa (1998a), char. 15; Costa (2004), char. 2) | + | + | 0 | 0 |
| Bifid epipleural ribs (vs. rod-shaped epipleural ribs) (Parenti (1981); Costa (1998a), char. 98; Costa (2009) char. 18) | 0 | + | 0 | 0 |
| Keel-shaped lateral process on middle part of terminal centrum (vs. smooth terminal centrum) (Costa (2012), char. 20) | 0 | + | 0 | 0 |
| Twisted and reduced lacrimal (vs. flat with wide posterior rim) (Parenti (1981); Costa (2004), char. 31) | 0 | + | + | + |
| Distinctive neural spine on first vertebra narrow or broad (vs. neural spine on first vertebra absent) (Costa (1990); Costa (1998b); Costa (2004); char. 34) | 0 | + | + | + |
| Shortened laminar proximal end of parhypural (vs. not reduced, overlapping terminal centrum) (Costa (2012), char. 12; Costa (2004), char. 39) | 0 | + | + | P |
| Long first dorsal fin ray attached to two proximal radials, preceded by one or two short fin rays (vs. single long first dorsal fin ray attached to two proximal radials) (Parenti (1981); Costa (1998a), char. 94; Costa (2004), char. 44) | 0 | + | + | + |
| Completely attached orbital rim (vs. ventrally attached) (Parenti (1981); Costa, (1998a), char. 103; Costa (2004), char. 58) | P | + | + | n.a. |
| Preural vertebra 2, haemal spine slightly wider than haemal spines of preural vertebrae 4 and 5 (vs. distinctively wider) (Costa (2012), char. 19; Costa (2004), char.43) (see Text) | 0 | + | + | P |
| Posterior border of distal portion of premaxilla straight (vs. indented) (Costa (1998a), char. 2; Costa (2004), char. 1) | 0 | 0 | + | + |
| Ventral process of maxilla bent (vs. straight to slightly curved) (Costa (1998a), char. 4) | 0 | 0 | + | n.a. |
| Coronoid process of dentary narrowed (vs. broad) (Costa (1998b), char. 13; Costa (2004), char. 5) | 0 | 0 | + | 0 |
| Ventral tip of autopalatinum not reaching the quadratum (vs. long, reaching quadratum) (Parenti (1981); Costa (1998a), char. 28; Costa (2004), char. 8) | 0 | 0 | + | 0 |
| Thin, C-shaped preoperculum (vs. robust and L-shaped preoperculum) (Costa (1990); Costa (1998a), char. 36; Costa (2004), char. 13) | 0 | 0 | + | 0 |
| Reduced uncinate process of third epibranchial (vs. elongate process) (Parenti (1981); Costa (1990); Costa (1998a), char. 56) | 0 | 0 | + | n.a. |
| Pronounced retrorse process of lateral ethmoid (vs. narrow, wide or absent) (Costa (1998a), char. 63) | 0 | 0 | + | n.a. |
| **Table 7. (Continued)** | | | | |
| Reduced lateral rim of frontal (vs. not reduced) (Costa (1998a), char. 69; Costa (2004), char. 29) | 0 | 0 | + | 0 |
| Minute dermosphenotic (vs. elongate or short) (Parenti (1981); Costa (1998a), | 0 | 0 | + | n.a. |

| | | | | |
|---|---|---|---|---|
| char. 73) | | | | |
| First postcleithrum absent (vs. present) (Parenti (1981); Costa (1990); Costa (1998a), char. 77) | 0 | 0 | + | n.a. |
| Branchiostegal and opercular membranes united (vs. separated) (Parenti (1981); Costa (1990); Costa (1998a), char. 102; Costa (1998b), char. 164; Costa (2004), char. 57) | 0 | 0 | + | 0 |
| Frontal scales arranged circularly around a central A-scale (vs. transversely arranged) (Parenti (1981); Costa (1998a), char. 104) | 0 | 0 | + | n.a. |

+, present; 0, absent; P, polymorphic; n.a., not applicable; ?, uncertain; Apl=Aplocheiloidei; †*Ken* =†*Kenyaichthys*; Noth, Nothobranchiidae; Riv, Rivulidae.

### 2.4.4. Polymorphism in †*Kenyaichthys*

†*Kenyaichthys* reveals a remarkable degree of polymorphism with regard to the character states of the parhypural, the arrangement of the proximal radials in the dorsal fin and the size dimensions of the hypural plate.

As described above, extant species of killifish can show considerable variation in their hypural plate dimensions (Tables 4 and S7). In contrast to the recent species, where the hypural plates are at least 4% of SL in length and 5% of SL in width, the here described fossils show smaller and sometimes also very tiny hypural plates (< 1.0% of SL, n = 8), which is a condition not found in any of the examined extant killifish specimens. In conclusion, the great size variation of the hypural plate in †*K. kipkechi* is higher compared to those of the extant species and may hint to the presence of more than one species, which, however, could not be confirmed based on other characters.

Two conditions of the proximal part of the parhypural are known. The plesiomorphic state is a parhypural that overlaps the terminal centrum and displays a well-developed hypurapophysis; this is the condition seen in the Aplocheilidae sensu strictu and in most cyprinodontoid families Costa (2004, 2012). The apomorphic state is a reduced parhypural that does not overlap with the terminal centrum and possesses a rudimentary hypurapophysis at most Costa (2004, 2012); this is the condition found in the Nothobranchiidae and Rivulidae, and also in the Cyprinodontidae, some Fundulidae, and most Goodeidae (all Cyprinodontoidei) (Costa, 2004, 2012). Among the specimens of †*Kenyaichthys kipkechi*, some specimens show a reduced parhypural (Figs. 2B1–2B2 and 9), while others have a parhypural that overlaps the terminal centrum (Fig. 11); the hypurapophysis is usually absent. Such intraspecific polymorphism of the parhypural character state has not previously been reported for any extant or fossil species of killifish.

In addition, most specimens of †*Kenyaichthys* reveal a parhypural, which is autogenous, but

in some specimens of †*K. kipkechi* the parhypural is fused to the ventral portion of the hypural plate to a variable extent (Table S6).

A comparable polymorphism regarding the parhypural is found in the atheriniform species *Pseudomugil signifer*, which shows an autogenous parhypural in the majority of the individuals, but a parhypural fused to the ventral portion of the lower hypural plate in some specimens (Saeed et al., 1989). The character " parhypural fused to the lower hypural plate" is consistently present only in the Melanotaeniidae and in some members of the Bedotiidae (see (Rosen, 1964; Collette, 1966; Allen, 1980; Parenti, 1981, 1993, 2008, 2014; Saeed, et al., 1989; Fujita, 1990; Stiassny, 1990; Dasilao and Yamaoka, 1998a; 1998b; Meisner, 2001; Sparks and Smith, 2004; Costa, 2012)), but it is difficult to discern the evolutionary state (apomorph or plesiomorph) of the character.

In the dorsal fins of killifishes, a single proximal radial (pterygiophore) generally supports each dorsal fin ray, but two proximal radials support the anteriormost long ray (regardless of whether preceding short rays are present or not). In some specimens of †*Kenyaichthys*, one or two short rays have been recognized, and the two proximal radials support the first long ray (Figs. 7A1–7A2 and 7D1–7D2), as in Nothobranchiidae and Rivulidae. In other specimens, however, the first or second short dorsal fin ray is supported by two proximal radials (Figs. 7B1–7B2 and 7C1–7C2). This condition is not known from any extant killifish species.

### 2.4.5. The species concept used for †*Kenyaichthys*

We found a distinct overlap between the meristic values of the described species, and meristic characters alone were not useful for species diagnosis. The high level of variation in meristic characters might be related to sexual dimorphism, as sexual dimorphism is usually present in killifishes (Parenti, 1981). For example, in some rivulid species (Austrolebias and Cynolebias), the males possess more rays in anal and dorsal fins than the females (Ahl, 1934; Weitzman and Wourms, 1967; Costa and Cheffe, 2005; Costa and Brasil, 2007; Ferrer et al., 2008).

Furthermore, we did not use differences in numbers of preural vertebrae to discriminate between species because this number can vary within a single species (this study and unpublished data of W. Costa, pers. communication, May 2013). While Costa (2012) assumed that cyprinodontiform species possess four to six preural vertebrae, our data derived from the

four species of *Aphanius* indicate that the number may be as low as three in some specimens (Table S7).

Also the hypural plate length and width were not considered as taxonomically meaningful characters for species discrimination in †*Kenyaichthys*, because intraspecific variation in hypural plate dimensions was also found in the examined extant killifish specimens and, furthermore, because the four closely related studied species of *Aphanius* displayed overlap in their hypural plate lengths and widths. A part of the variation seen within the individual hypural plate dimensions may perhaps reflect sexual dimorphism since the males of the four species of *Aphanius* studied here showed higher mean values for the length and width of the hypural plates than the females. Such a sexual dimorphism in the hypural plate size could result from different swimming activity in females and males, because aggressive behaviour of males during courtship is known for *Aphanius* and several other killifish species (Haas, 1976; Kodric-Brown and Mazzolini, 1983; Fuller, 2001; Edenbrow and Croft, 2012). A larger hypural plate probably helps to create a more effective tail strike during "tail beating" behaviours of territorial males, as in *Fundulus waccamensis* (Shute et al., 1983) or *Cyprinodon macularius* (Barlow, 1961). At the same time, a bigger hypural plate might impair the swimming performance of the males, because of higher drag, as in *Poecilia reticulata* (Karino et al., 2006).

### 2.4.6. Taxonomic implications: Does †*Kenyaichthys* represent a species flock?

One possible explanation for the huge intraspecific variation seen in †*K. kipkechi* is that †*K. kipkechi* may contain several species "*in statu nascendi*" (Villwock, 1994) or might represent a species flock. The differentiation between a species *in statu nascendi* and a "real" species is based on the degree of sexual isolation; species *in statu nascendi* are located between complete panmixis and complete sexual isolation. Examples include the *Aphanius anatoliae* and the *Cyprinodon variegatus* group (Villwock, 1983), the individual populations of which are easily distinguishable in their external morphology, but reveal a gradient in their degree of hybrid sterility and sexual isolation. A species flock, on the other hand, is a monophyletic group of closely related species coexisting in the same area (Greenwood, 1984; Mayr, 1984) such as the species of *Cyprinodon* in Laguna Chichancanab in Mexico (Horstkotte and Strecker, 2005), or the littoral species of *Orestias* in Lake Titicaca in Peru (Parenti, 1984;

Northcote, 2000). The only fossil species flock known from Africa is the cichlid species flock of *Mahengechromis* from the Eocene lake Mahenge (Murray, 2000).

We consider the concept of the species flock to be quite applicable to †*Kenyaichthys*. The species of the modern *Orestias*- and *Cyprinodon* species flocks, like the fossil species studied here, show a high level of overlap in their meristics and morphometrics (Parenti, 1984; Strecker, 2002). In the case of the *Cyprinodon* species flock, a single species (*C. maya*) was found to be sexually isolated, whereas the remainder exhibited different grades of hybridization and represent different evolutionary stages (Horstkotte and Strecker, 2005). Horstkotte and Strecker (2005) assumed that the flock evolved due to adaptive radiation because of trophic differentiation and in the absence of competitors. A further report with similar implications is the study on Nicaraguan Midas cichlids (*Amphilophus* cf. *citrinellus*) from the Crater Lake Apoyo (Geiger et al., 2013). The authors identified six species with different levels of reproductive isolation and interpreted them as a species flock *in statu nascendi*. Based on the level of overlap in morphometric and meristic characters we assume that the assemblage of the specimens of †*Kenyaichthys* also represents a species flock *in statu nascendi*.

### 2.4.7. Environmental implications

Previous paleoenvironmental reconstructions for the Lukeino area suggest freshwater conditions and no environmental disturbances (Pickford, 1975; Pickford et al., 2009). However, the scarcity of typical freshwater fishes such as cyprinids in our samples indicates that some environmental factors prohibited the presence of other fish species. The most likely explanation is that seasonal aridity, which has been reported for the Late Miocene of Eastern Africa based on (amongst others) palynological and paleobotanical remains and changes in herbivorous mammal diversity (Bobe, 2006; Jacobs et al., 2010), increased the salinity from time to time, and may eventually have led to episodes of drought. Only extremely euryhaline and eurytherm fish species that are capable of producing eggs that are resistant to drying can survive such adverse periods. Indeed, some genera of the Nothobranchiidae and Rivulidae provide modern examples of such species (Murphy and Collier, 1997), and perhaps some fossil groups of killifishes, maybe even the †Kenyaichthyidae discussed here, possessed such survival skills.

It appears that †*Kenyaichthys* was well adapted to its environment. This is additionally supported by the relatively low incidence of supernumerary spines (25% of 127 specimens of †*Kenyaichthys*, in which this character could be examined) in the caudal skeleton, which is comparable to that seen in hatchery-reared fish species (23%) (Bensimnon-Brito et al., 2012), but higher compared to species living in pristine natural environments (12%) (Bogutskaya et al., 2011). In the case of environmental pressures, a relatively higher percentage of caudal skeletons with supernumerary spines would be likely. This phenomenon is usually explained by the fusion of two vertebral centra owing to unfavourable conditions such as vitamin C deficiency, excess supply of vitamin A, or parasite infection (Dedi et al., 1995; Hosoya and Kawamura, 1998; Madsen and Dalsgaard, 1999; Kvellestad et al., 2000; Gavaia et al., 2002).

An additional hint to some environmental disturbances is the hunchback curvature of the vertebral column in 50% of †*Kenyaichthys*, as described above. This abnormality can be provoked by elevated concentration of heavy metals such as cadmium, copper and zinc or significant variations of environmental parameters such as temperature (Bengtsson et al., 1975; Bengtsson et al., 1988; Gorman and Breden, 2007; Messaoudi et al., 2009a; Messaoudi et al., 2009b). The percentage of specimens of †*Kenyaichthys*, in which such a vertebral deformity is present, is more than three times higher compared to reports on spinal deformities in polluted waters (15.63– 17.67% polluted vs. 1.96– 4.58% non-polluted (Messaoudi et al., 2009a; Messaoudi et al., 2009b)). However, the extent of the hunchback seen in the fossils (Fig 5A and 5C) is less extreme than seen in extant specimens (see Messaoudi et al., (2009a, b) pages 363 and 554).

Spinal deformations in extant specimens are usually explained by the adverse influence of zinc and copper, which impair the neuromuscular system (Messaoudi et al., 2009b). Moreover cadmium can disrupt the calcium metabolism, resulting in hypocalcaemia and destabilization of bones (Kessabi et al., 2009). The natural source of zinc, copper and cadmium is weathering of rocks and soil (Shaw, 1989), as well as volcanic emissions (WHO, 2000; Kumar and Singh, 2010). As the Lukeino area was influenced by volcanic activity (Pickford, 1978), the most likely explanation for the vertebral deformations in †*Kenyaichthys* is that the aquatic environment was in close proximity to an active volcano delivering ashes into the water.

### 2.4.8. Biogeographic implications

The vicariance hypothesis and the dispersalism theory describe the evolutionary history of the killifishes in two different ways. According to the vicariance hypothesis, the Cyprinodontiformes could be found on the whole continent of Gondwana during the Cretaceous, and their present-day distribution is mainly due to the later break-up of the super-continent (Parker and Kornfield, 1995; Murphy and Collier, 1997). In contrast to this, the dispersalists argue that the Neotropics bear the highest generic diversity and therefore should be taken as the centre of origin of all Cyprinodontiformes, from where they spread out during the middle or late Cretaceous by crossing marine waters, as most cyprinodontoids and some aplocheiloids are considered to be secondary freshwater fish (Lundberg, 1993; Briggs, 2003). However, there is no evidence for fossil Aplocheiloidei prior to †*Kenyaichthys* (Late Miocene), whereas the Cyprinodontoidei have a good fossil record since the Paleocene. †*Kenyaichthys* currently is the only and oldest representative of a fossil Aplocheiloidei, but future findings of fossil Aplocheiloidei are necessary to understand whether the vicariance or the dispersalism model provide a reliable scenario for the evolutionary history of the killifishes.

## 2.5. Conclusion

The here studied fish fossils from the Upper Miocene Lukeino Formation document the first appearance of representatives of the Aplocheiloidei in the fossil record, which we assign to † Kenyaichthyidae nov. fam. and †*Kenyaichthys* nov. gen. †*Kenyaichthys* shows remarkable polymorphism of the hypural plate dimensions, the parhypural and the dorsal fin pterygiophores and displays a combination of apomorphic characters that is not diagnostic for any of the extant aplocheiloid families. It appears that †*Kenyaichthys* was an annual fish that belonged to an ancient clade that was related to the present-day lineage of the Nothobranchiidae.

Patterns of variation in neural and haemal spine dimensions in the caudal vertebrae of †*Kenyaichthys* and the extant species studied here indicate that previously described synapomorphies for the Cyprinodontoidei (i.e. **"**neural and haemal spines of PU3 wider than spines of preural vertebrae anterior to PU4 vs. about equal**"**) and the

Nothobranchiidae+Rivulidae (i.e. **"**haemal spine of PU2 slightly wider than haemal spines of PU4 and PU5 vs. distinctively wider**"**) need to be revised.

The here described new species †*Kenyaichthys kipkechi* most likely represents an ancient killifish species flock *in statu nascendi*. This indicates that species flocks in the fossil record, which have only rarely been recognized in previous work, may not be as exceptional as previously thought. Such knowledge is essential for a better understanding of the species diversity in the fossil record.

The presence of a killifish assemblage in the Lukeino Formation, while typical freshwater fish are extremely rare, is not in conflict with the reconstruction of the Lukeino area as a freshwater-dominated environment, but indicates an environment in the Late Miocene of Eastern Africa that was influenced by seasonal aridity.

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## Supporting Information

The following documents can be retrieved from the webpage of PLOS ONE at http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0123056#sec051

**S1 Table. Morphometric characters of †Keny*aichthys kipkechi* sp. nov., given in mm (top) and in % of SL (below).**

**S2 Table. Measurements of the premaxilla and maxilla of †*Kenyaichthys kipkechi* sp. nov.**

**S3 Table. Meristic values of all specimens of †*Kenyaichthys* gen. et sp. nov.**

**S4 Table. Spine-ratios of †*Kenyaichthys* gen. et sp. nov.**

**S5 Table. Dimensions of key scales of four specimens of †*Kenyaichthys* gen. et sp. nov.**

**S6 Table. Polymorph characters of †*Kenyaichthys* gen. et sp. nov. and detectability of the neural spine on the first vertebra (NS 1).**

**S7 Table. Hypural plate length (lH) and width (wH), and numbers of preural vertebrae obtained from the extant cyprinodontoid and aplocheiloid specimens used for comparison.**

**S8 Table. Spine-ratios of the recent cyprinodontoid and aplocheiloid specimens used in this study.**

**S9 Table. Description of characters used for phylogenetic analysis, and distribution of character states.** Characters are compiled from the following literature (see text for details).

**S10 Table. Character-taxon matrix used in the phylogenetic analysis shown in Figs 14 and 15 based on 72 characters of 13 terminal taxa and two outgroups.**

**S1 NEXUS File.**

**S1 Fig. Measurements of spines. A** on a rounded tip; **B** on a blunt tip; **C** on spines with tip not covered by caudal fin rays and on spine covered by fin rays.

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# 3. †*Protochromis pickfordi* gen. et sp. nov. from the upper Miocene – a precursor lineage of modern Lake Tanganyika cichlids?

Melanie Altner, *, [1] Ulrich K. Schliewen, [2] and Bettina Reichenbacher [1]

[1]Department of Earth and Environmental Sciences, Paleontology & Geobiology, Ludwig-Maximilians Universität München, Richard-Wagner-Strasse 10, DE 80333 Munich, Germany, m.altner@lrz.uni-muenchen.de, b.reichenbacher@lrz.uni-muenchen.de;

[2] SNSB Bavarian State Collection of Zoology, Department of Ichthyology, Münchhausen-Strasse 21, DE 81247 Munich, Germany, Schliewen@zsm.mwn.de

Note: This article is currently under revision and the name of the new genus will be changed.

**Abstract**

Cichlidae represent a highly diverse group of tropical freshwater fishes. However, their sparse fossil record complicates exploration of their evolutionary history. Here we present a new fossil cichlid from the upper Miocene (9-10 Ma) of the Ngorora Fish Lagerstätte in Central Kenya (East African Rift System, EARS) and infer its phylogenetic relationships based on a comprehensive comparative dataset comprising meristic and osteological data from all present-day tribes from Lake Tanganyika plus several riverine cichlids. †*Protochromis pickfordi* gen. et sp. nov. displays a unique combination of characters, including six lateral line tubules on the lacrimal, a third lateral line segment, cycloid scales and low numbers of vertebrae (28) and dorsal fin spines and rays (XIII/9). It cannot be attributed to any previously described taxon of fossil cichlids from Africa, the Arabian Peninsula or Europe. However, comparative morphology reveals affinities with the Lake Tanganyika tribes Limnochromini and Ectodini, and a Principal Coordinates Analysis (PcoA) based on our meristic data supports this placement. We propose that the new fossil may represent a proto-Tanganyika lineage which is closely related to the precursor lineage of the modern tribes of 'ancient Tanganyika mouth-brooders'. As Lake Tanganyika is located in the western branch of the EARS, the discovery of the new fossil taxon in its eastern branch (Central Kenya Rift) supports the recently proposed 'melting-pot Tanganyika hypothesis'. This posits that the cichlids of modern Lake Tanganyika are derived from riverine lineages that had already undergone cladogenesis prior to its formation.

## 3.1 Introduction

Cichlidae are an extremely diverse tropical family of mainly freshwater fishes, comprising some 220 genera and at least 1400 species (Kolm et al., 2006; Fitzsimmons and Watanabe, 2010). Based on morphological and molecular data, four subfamilies can be recognized: Cichlinae (distributed in South, Central and North America), Etroplinae (restricted to South Asia and Madagascar), Pseudocrenilabrinae (restricted to Africa and the Middle East), and Ptychochrominae (limited to Madagascar) (Cichocki, 1976; Stiassny, 1991; Chakrabarty, 2004; Sparks and Smith, 2004; Smith et al., 2008). Since Cichlidae are extraordinarily diverse, often highly specialized in ecology and behavior (e.g. parental care, mouthbrooding), and show a broad spectrum of trophic adaptations, they represent one of the most intensively studied fish groups, and have long been used as model organisms for the study of speciation and adaptive evolution (e.g. Kocher, 2004; Seehausen, 2006; Genner et al., 2007). However, their fossil record is scanty and this makes it difficult to fully explore their evolutionary history.

Pseudocrenilabrinae (African cichlids) represent the most species-rich subfamily of the Cichlidae. Based on molecular phylogenetics, they can be separated into 27 lineages comprising around 150 genera (Stiassny et al., 2007; Schwarzer et al., 2009; Dunz and Schliewen, 2013; Loh et al., 2013; Weiss et al., 2015). According to these molecular studies, the earliest diverging lineages of the Pseudocrenilabrinae include the Heterochromini, Tylochromini, Chromidotilapiines, Hemichromines and Pelmatochromines, while Haplotilapiines represent the youngest major split. The Haplotilapiines are made up of a total of 22 lineages, among which the ‘East African Radiation’ (EAR) is the most speciose monophyletic subclade.

The cichlids assigned to the EAR are characterized by an enormous capacity for rapid speciation and adaptive radiations, and are mainly distributed in the Great Lakes of the Rift Valley - Tanganyika, Malawi and Victoria (e.g. Meyer et al., 1990; Sturmbauer and Meyer, 1993; Moran et al., 1994; Sturmbauer et al., 1994; Loh et al., 2013). Three out of the 13 groups of the EAR are widespread across East and Central Africa (Lamprologini, Orthochromines, Haplochromini (including Tropheini)), while the remaining ten tribes (Boulengerochromini, Bathybatini, Trematocarini, Benthochromini, Cyprichromini, Perissodini, Cyphotilapiini, Limnochromini, Ectodini, Eretmodini) are endemic to the Lake Tanganyika drainage and represent the most ancient EAR lineages. According to Weiss et al.

(2015), the Tanganyika lineages can be further subdivided into four major groups: (i) the 'most ancient Tanganyika tribes' (Boulengerochromini, Bathybatini, Trematocarini), (ii) Lamprologini and Eretmodini, (iii) the 'ancient Tanganyika mouth-brooders' (Benthochromini, Cyprichromini, Perissodini, Cyphotilapiini, Limnochromini, Ectodini), and (iv) the Tropheini, a subgroup of the Haplochromini.

The megadiversity of the Pseudocrenilabrinae contrasts with their comparatively meagre fossil record. Cichlid fossils from Africa and Arabia include some completely preserved skeletons, but most are represented by isolated teeth, bones, vertebrae and scales. In all, a total of 17 fossil cichlid taxa have been described from Eocene to Pliocene sediments of Africa and Arabia on the basis of articulated skeletons, but their phylogenetic position has not been fully clarified to date (Table 1). The objective of this study is to describe a newly discovered fossil cichlid specimen from the upper Miocene Ngorora Formation (Central Kenya, East African Rift system) and to infer its phylogenetic position among modern African cichlids based on a comprehensive comparative dataset comprising meristic and osteological data from all present-day Lake Tanganyika tribes, as well as several riverine forms which might be related to the cichlid fauna of Lake Tanganyika.

**Table 1. Eocene to Pliocene fossil cichlid species from Africa and Arabia that are based on articulated skeletons and their putative phylogenetic placement according to the literature.** Extinct species are marked with †.

| Proposed assignment of previous authors | Fossil cichlid species | Eocene | Oligocene | lower Miocene | Middle – upper Miocene | Upper Miocene | Lower Pliocene |
|---|---|---|---|---|---|---|---|
| Murray, 2000; fig. 11: Based on the cladogram of Lippitsch (1995, 1998) near to Cyprichromini (= EAR tribe); Murray, 2001; fig. 3: Not resolved | †*Mahengechromis brachycranium* Murray 2000 | ~46 Ma | | | | | |
| | †*Mahengechromis curvifrons* Murray 2000 | | | | | | |
| | †*Mahengechromis ellipticus* Murray 2000 | | | | | | |
| | †*Mahengechromis plethos* Murray 2000 | | | | | | |
| | †*Mahengechromis rotundus* Murray 2000 | | | | | | |
| Lippitsch & Micklich, 1998: Heterochromini | ?*Heterochromis* sp. Lippitsch & Micklich 1998 | | 33.9–23.0 Ma | | | | |
| Lippitsch & Micklich, 1998: Close to Tilapiini | Tilapiini Group 2 Lippitsch & Micklich 1998 | | | | | | |
| Lippitsch & Micklich, 1998: Haplochromine assemblage | Tilapiini Group 3 Lippitsch & Micklich 1998 | | | | | | |
| Van Couvering, 1982: Close to *Pelmatochromis* or *Paratilapia* | †*Macfadyena dabanensis* Van Couvering 1982 | | | | | | |
| Van Couvering, 1982: Close to Tropheini | †*Kalyptochromis hamulodentis* Van Couvering 1982 | | | | | | |
| Van Couvering, 1982: Close to Haplochromini, *Cichla* or *Hemichromis* | †*Nderechromis cichloides* Van Couvering 1982 | | | 23.0–16.0 Ma | | | |
| Van Couvering, 1982: Close to *Pelmatochromis* | †*Palaeofulu kuluensis* Van Couvering, 1982 | | | | | | |
| Van Couvering, 1982: Oreochromini | †*Oreochromis martyni* (Van Couvering 1982) | | | | 16.0–5.3 Ma | | |
| Van Couvering, 1982: Close to *Pelmatochromis* or *Tilapia* or Haplochromini | †*Palaeochromis darestei* Sauvage 1907 | | | | | 11.6–5.3 Ma | |
| | †*Palaeochromis rouselleti* Sauvage 1907 | | | | | | |
| Carnevale et al., 2003: Oreochromini | †*Oreochromis lorenzoi* Carnevale et al. 2003 | | | | | | |
| Murray & Stewart, 1999: Oreochromini | †*Oreochromis harrisae* Murray & Stewart 1999 | | | | | | 3.6–2.6 Ma |

### 3.1.1 Geological setting

**Study site.** The single specimen with which this study is concerned was collected at the Waril site (0°40´56.21´´N 35°43´7.43´´E), located in the Kerio Valley, to the west of the Tugen Hills in the Central Kenya Rift Valley (Fig. S1). The fish-bearing sediments are tuffaceous lacustrine siltstones. Waril is part of the Ngorora fish Lagerstätte and can be assigned to the upper Miocene (9-10 Ma) based on lithostratigraphy, and on the presence of an equid in a paleosol above the lacustrine sediments (Pickford, 1978; Jacobs, 2002; Rasmussen et al., 2015). Ancient Lake Waril was a comparatively deep and alkaline lake, and the climate in the area was seasonally dry (Pickford, 1978; Jacobs, 2002; Kingston et al., 2002; Tiercelin and Lezzar, 2002; Bonnefille, 2010; Bamford et al., 2013; Rasmussen et al., 2015).

## 3.2. Material and Methods

### 3.2.1. Fossil material

The material consists of a single skeleton in part and counterpart (OCO-5-22/OCO-5-35). It is deposited in the Museum in Kipsaraman, Kenya, which is affiliated with the National Museum in Nairobi. Prefix OCO for Orrorin Community Organization.

### 3.2.2. Comparative material

Our newly assembled comparative dataset comprises 116 species (50 genera, 17 lineages, 455 specimens) representing almost all genera of all present-day Lake Tanganyika tribes (according to Schwarzer et al., 2009; Dunz and Schliewen, 2013; Weiss et al., 2015), plus species of *Orthochromis* Greenwood 1954 (tribe Haplochromini) and *Haplochromis vanheusdeni* Schedel, Friel & Schliewen 2014 (Appendix 1). The ‘most ancient Tanganyika mouth-brooders’ (sensu Weiss et al., 2015) are represented by all genera and all species, with the sole exception of *Trematocara caparti* Poll 1948. The same is true for the Eretmodini, the exception here being *Eretmodus marksmithi* Burgess 2012. The dataset for the Lamprologini is incomplete, but all major groups (all genera) are included. Furthermore, the dataset contains all genera of the ‘ancient Tanganyika mouth-brooders’ (sensu Weiss et al., 2015) with the exception of *Baileychromis* Poll 1986.

Members of the genus *Orthochromis* were included in our analyses because of their potential role as contributors to the Lake Tanganyika radiation (Meyer et al., 2015; Weiss et al., 2015). The species of *Orthochromis* are a riverine cichlid group, which is unlikely to be

monophyletic and can be further subdivided into five lineages according to their geographic origin, i.e. (i) Malagarasi-*Orthochromis* from the rivers Malagarasi, Luiche and Rugufu, (ii) LML-*Orthochromis* from the Luapula-Mweru system and the Lualaba/Congo mainstream, (iii) Northern Zambian-*Orthochromis* from Northern Zambia, (iv) *Orthochromis torrenticola* from the Democratic Republic of Congo, and (v) *Orthochromis machadoi* from the Cunene in Angola (Weiss et al. 2015, and unpublished data of UKS). Moreover, *Haplochromis vanheusdeni* Schedel, Friel & Schliewen 2014 was included because it closely resembles *Orthochromis* cichlids, and because its phylogenetic position is as yet undetermined (Schedel et al., 2014).

**Note.** We follow previous authors in using the suffix '-ines' in informal group names and the suffix '-ini' when referring to formal tribes (see Schwarzer, 2011).

### 3.2.3. Morphological analyses

Adherent sediment particles were carefully removed from the fossil skeleton using a needle. Osteological, meristic and morphometric characters of the fossil were examined using a stereomicroscope equipped with a digital camera. SEM images of teeth were prepared using a LEO 1430VP at 15 kV, and picture quality was enhanced using Photoshop CS6. Radiographs were produced for each individual included in the comparative dataset using a Faxitron UltraFocus LLC x-ray unit, and served as the basis for investigation of the osteological and meristic characters of each specimen. Morphometric measurements and meristic counts follow Holčík (1989) and Barel et al. (1977). Measurements were taken to the nearest 0.01 mm and counts of vertebrae exclude the terminal centrum. Dorsal and anal fin ray counts included every discernible ray, regardless of whether or not it was associated with a pterygiophore. Measurements on the fossil were standardized based on the body length, i.e. the distance from the posterior margin of the operculum to the posterior margin of the hypural plate. Interpretation of osteological characters follows Van Couvering (1982), Poll (1986), Kullander (1998), Lippitsch (1995, 1998) and Takahashi (2003a, 2003b).

### 3.2.4. Statistical analyses

We conducted a Principal Coordinates Analysis (PCoA) in the program PAST 3.10 (Hammer et al., 2001) based on the characters noted in the fossil and inspection of the same characters

in the comparative dataset (i.e. cichlids of all present-day Lake Tanganyika tribes plus *Orthochromis* (tribe Haplochromini) and *Haplochromis vanheusdeni*, see above). Characters used as variables included counts of spines and rays in the dorsal and anal fins, counts of abdominal, caudal and total vertebrae, number of predorsals and the position of the vertebra associated with the pterygiophore of the last dorsal fin spine. The PCoA is a statistical tool for multivariate analysis, which visualizes group differences, as well as individual outliers based on similarities or dissimilarities in the dataset. Unlike Principal Components Analysis (PCA), PCoA deals with qualitative data and requires no *a priori* assumptions; furthermore, PCoA yields more reliable results in the case of missing data (Hammer and Harper, 2006; Leyer and Wesche, 2007).

**Institutional abbreviations.** AMNH, American Museum of Natural History; BMNH, Natural History Museum, London; CU, Cornell University Museum of Vertebrates; IRSNB, Institut Royal des Sciences Naturelles de Belgique, Bruxelles; MNHN, Muséum national d' Histoire naturelle, Paris; MRAC, Royal Museum for Central Africa, Tervuren; NMW, Naturhistorisches Museum, Wien; NRM, Naturhistoriska riksmuseet, Stockholm; RG, Royal Museum for Central Africa; ROM, Royal Ontario Museum; SAIAB, South African Institute for Aquatic Biodiversity; SMF, Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt; ZSM, Bavarian State Collection of Zoology, Munich.

## 3.3. Systematic Paleontology

CICHLIDAE Bonaparte, 1835

PSEUDOCRENILABRINAE Fowler, 1935

HAPLOTILAPIINES Schliewen and Stiassny, 2003

†*Protochromis* nov. gen.

**Generic Diagnosis.** Slender cichlid of small size with a tripartite lateral line system; a lacrimal with lateral line branched into six tubules; a unique combination of meristic characters including 28 (15+13) vertebrae, dorsal fin formula XIII/9 and anal fin formula III/9; cycloid scales and approximately 30 scales in the longitudinal line.

**Etymology.** From the Greek terms ‘prôtos’ meaning ‘early form of’, and ‘chromis’ which is a widely used suffix for cichlid species. These terms refer to the status of the new genus as a potential precursor lineage of modern haplotilapiine cichlids.
**Type species.** †*Protochromis pickfordi* sp. nov.

†*Protochromis pickfordi* sp. nov.

(Figs. 1–5)

**Holotype.** OCO-5-22/35, partially complete skeleton in part and counterpart (Fig. 1A1–A3), approximately 60 mm total length, 33.5 mm body length.
**Diagnosis.** As for genus.
**Etymology.** Species named in honor of the distinguished paleontologist Martin Pickford in recognition of his outstanding contribution to the geology and paleontology of East Africa.
**Locality, horizon and age.** Outcrop Waril (0°40´56.21´´N 35°43´7.43´´E) in Central Kenya; Ngorora Formation, member E; late Miocene (9-10 Ma) (see Rasmussen et al., 2015).

**General description.** Measurements of the holotype are summarized in Table 2.
Body preserved in lateral view, elongate to torpedo-shaped and laterally compressed (Fig. 1A1–A3). Head incomplete, preserved in dorsolateral view; lacrimal with lateral line branched into six tubules. Scales large and cycloid: tripartite lateral line (Figs. 2A1–B2, C).
**Neurocranium.** Nasals, frontals and the orbit are not preserved, occipital region severely crushed. Imprints of oval sagittal otoliths displaying a prominent rostrum and a small antirostrum are recognizable (Fig. 1A1–A3). The pterotic is partly preserved and shows canals that probably represent the neurocranial lateral line canals (NLC) (Fig. 1A1–A3). The lacrimal shows the lateral line branching into six tubules, and is almost as deep as it is wide (2.47 x 2.79 mm), its ventral and posterior outline is convex, the dorsal and anterior contours are rather straight (Figs. 1A1–A3, 3A1–A2).
**Jaws and teeth.** Approximately 130 slender teeth are present, ranging in size from 0.21 to 0.23 mm length and 0.03 to 0.06 mm width. Some are unicuspid and hook-like with a shoulder, others are simple unicuspid (Fig. 3C–F). Based on their positions between the supracleithrum and the first vertebra, we consider these teeth to be pharyngeal. No bicuspid or tricuspid teeth are preserved. Jaw teeth and bones are not preserved.

**Suspensorium and hyoid arches.** The large and approximately triangular operculum is robust, its dorsal, anterior and posterior borders are convex, the articulation process is small (both parts; Fig. 1A1–A3) and lacks scales. The posterior part of a large and rounded suboperculum is visible below the operculum (Fig. 1A1–A3). Other bones of the suspensorium are not preserved.

**Table 2. Morphometric measurements and meristic counts of †*P. pickfordi*.**

| Character | mm | % of BL |
|---|---|---|
| Body length | 33.47 | – |
| Head length | – | – |
| Head height | – | – |
| Maximum body height | 12.50 | 37.3 |
| Maximum body height at anal fin origin | 10.55 | 31.5 |
| Length of dorsal fin base | 23.04 | 68.8 |
| Length of dorsal fin spine 1 | 1.98 | 5.9 |
| Length of dorsal fin spine 2 | 3.80 | 11.4 |
| Length of dorsal fin spine 3 | 4.37 | 13.1 |
| Length of dorsal fin spine 4 | 5.41 | 16.2 |
| Length of dorsal fin spine 5 | 5.47 | 16.3 |
| Length of dorsal fin spine 6 | 5.45? | 16.3 |
| Length of dorsal fin spine 7 | 5.81? | 17.4 |
| Length of dorsal fin spine 8 | – | – |
| Length of dorsal fin spine 9 | – | – |
| Length of dorsal fin spine 10 | 5.91 | 17.7 |
| Length of dorsal fin spine 11 | 6.15 | 18.4 |
| Length of dorsal fin spine 12 | 6.41 | 19.2 |
| Length of dorsal fin spine 13 | 7.02 | 21.0 |
| Length of anal fin base | 6.14 | 18.3 |
| Length of anal fin spine 1 | 4.04 | 12.1 |
| Length of anal fin spine 2 | 5.35 | 16.0 |
| Length of anal fin spine 3 | 6.83 | 20.4 |
| Length of pectoral fin | – | – |
| Length of pectoral fin base | – | – |
| Length of pelvic fin | 6.61 | 19.7 |
| Length of pelvic fin base | 1.85 | 5.5 |
| Distance between pectoral fin base and anal fin base | – | – |
| Distance between pelvic fin base and anal fin base | 14.49 | 43.3 |
| Distance between pectoral fin base and pelvic fin base | – | – |
| Minimum body height | 4.48 | 13.4 |
| Length of caudal fin | 10.65 | 31.8 |
| Length of caudal peduncle | 10.95 | 32.7 |
| Meristics | | |
| Dorsal fin | XIII/9 | |
| Anal fin | III/9 | |
| Pectoral fin | 9+ | |
| Pelvic fin | I/5 | |
| Caudal fin | 7+8+8+7 | |

## 3. †Protochromis pickfordi gen. et sp. nov. from the upper Miocene – a precursor lineage of modern Lake Tanganyika cichlids?

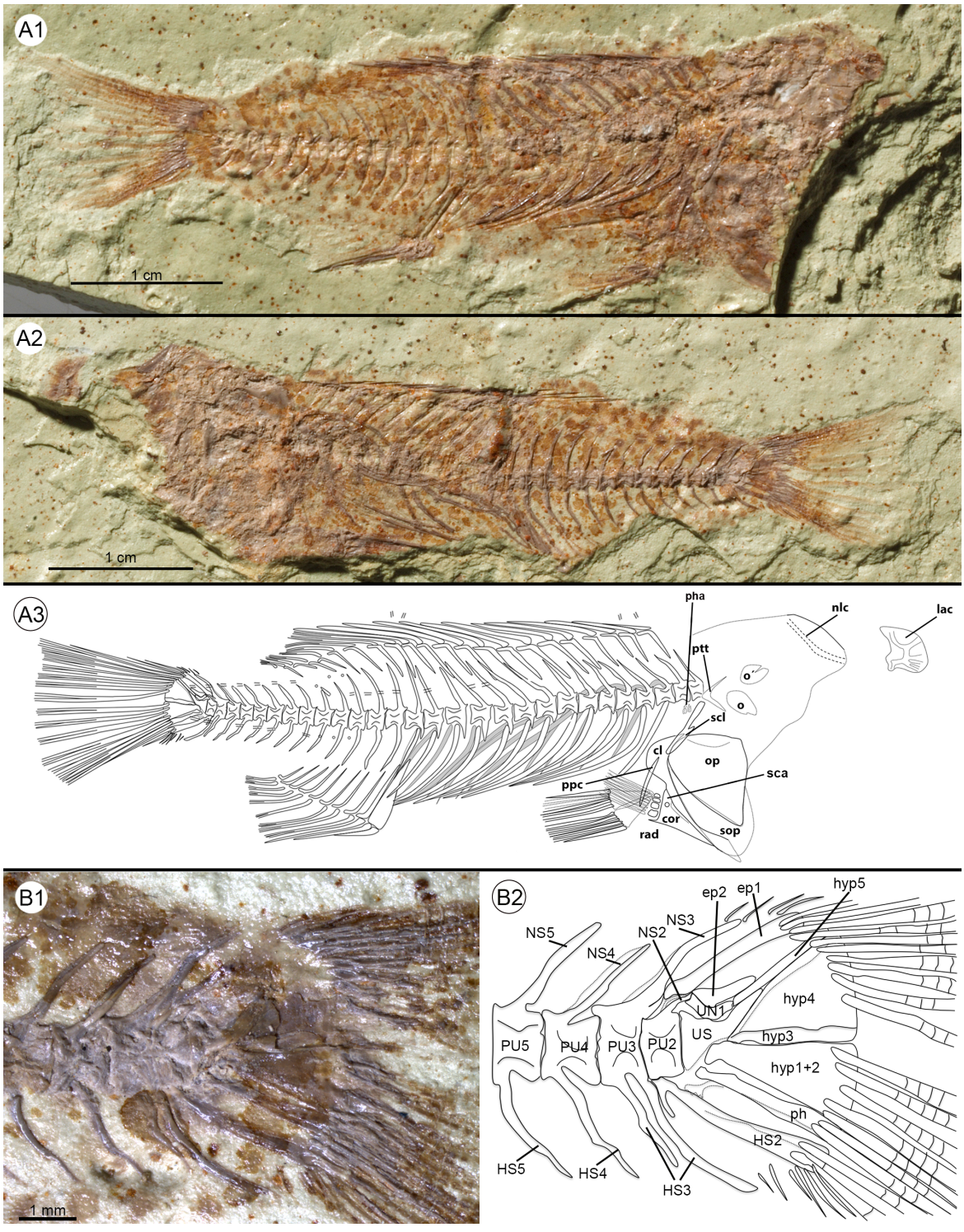


**Figure 1.** †***Protochromis pickfordi*** **gen. et sp. nov. A1–A2**, Holotype in part (OCO-5-35) and counterpart (OCO-5-22); **A3**, Right lateral view of the specimen (shading refers to ribs from the left side of the specimen); **B1**, Caudal skeleton of †*Protochromis pickfordi* gen. et sp. nov. (OCO-5-22); **B2**, Reconstruction of caudal skeleton in left lateral view. **Abbreviations**: **cl**, cleithrum; **cor**, coracoid; **ep**, epural; **hs**, haemal spine; **hyp**, hypural plate; **lac**, lacrimal; **nlc**, neurocranial lateral line canal; **ns**, neural spine; **o**, otolith; **op**, operculum; **ph**, parhypural; **pha**, pharyngeal teeth; **ppc**, postcleithrum; **ptt**, posttemporal; **pu**, preural centrum; **rad**, radials; **sca**, scapula; **scl**, supracleithrum; **sop**, suboperculum; **us**, urostyle; **un1**, uroneural 1; =, tubular lateral line scale; °, pitted lateral line scale.

## 3. †Protochromis pickfordi gen. et sp. nov. from the upper Miocene – a precursor lineage of modern Lake Tanganyika cichlids?

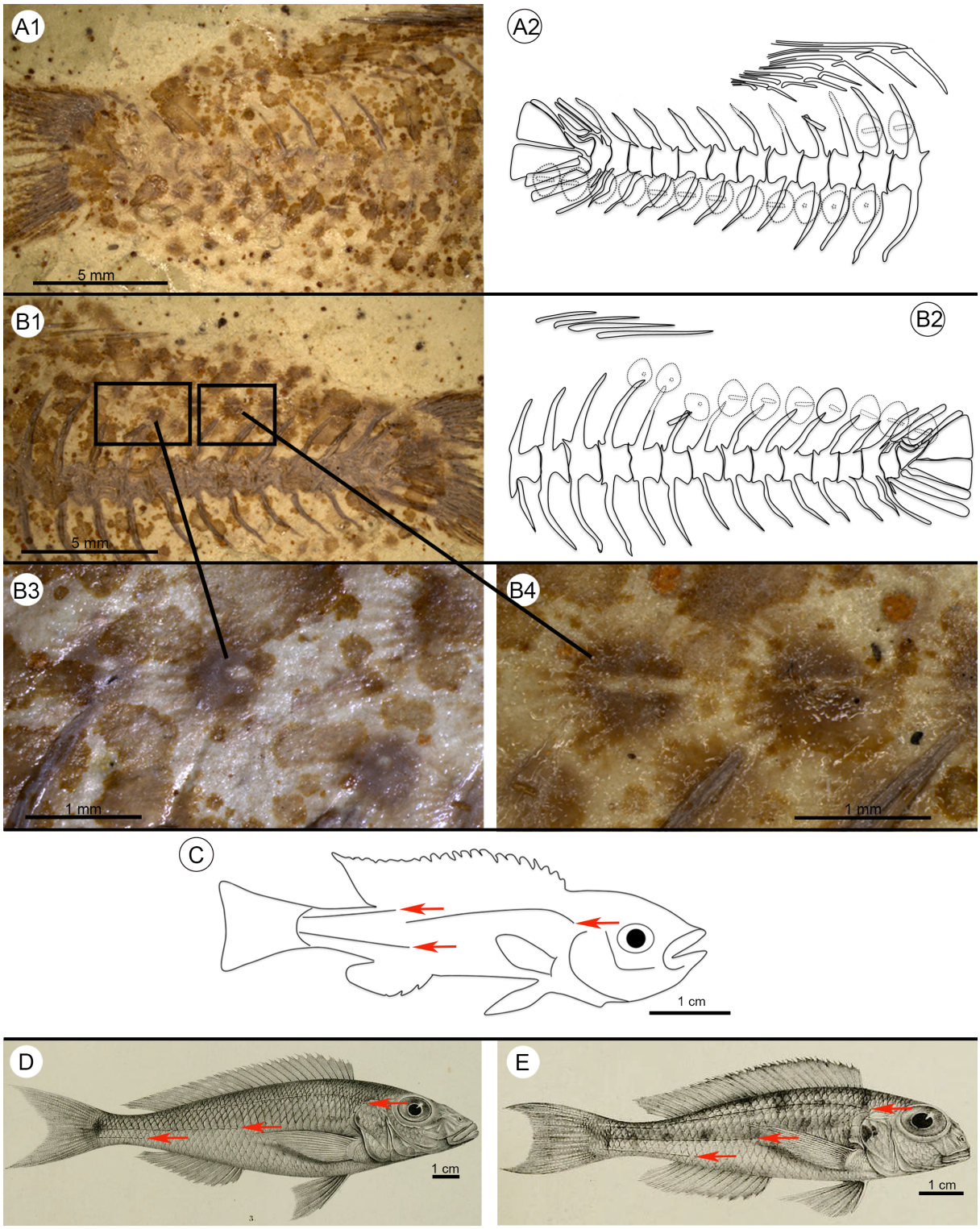


**Figure 2. A1–C**, Part and counterpart of the caudal portion and complete reconstruction of †*Protochromis pickfordi* gen. et sp. nov., showing the pattern of the three lateral line (trunk canal) segments; **A1–A2**, Segment of the posterior trunk canal extending below the vertebral column and also below the anterior canal segment (OCO-5-35); **B1–B2**, Additional segment of the posterior trunk canal above the vertebral column (OCO-5-22); **B3–B4**, Close-up views of pitted and tubular lateral line scales; **C–E**, tripartite lateral line pattern in Ectodini and the new fossil cichlid: **C**, †*Protochromis pickfordi* gen. et sp. nov.; **D**, *Grammatotria lemairii*; **E**, *Xenotilapia sima*. Arrows indicate the three trunk canal segments. Panels **D** and **E** modified from Pellegrin (1904).

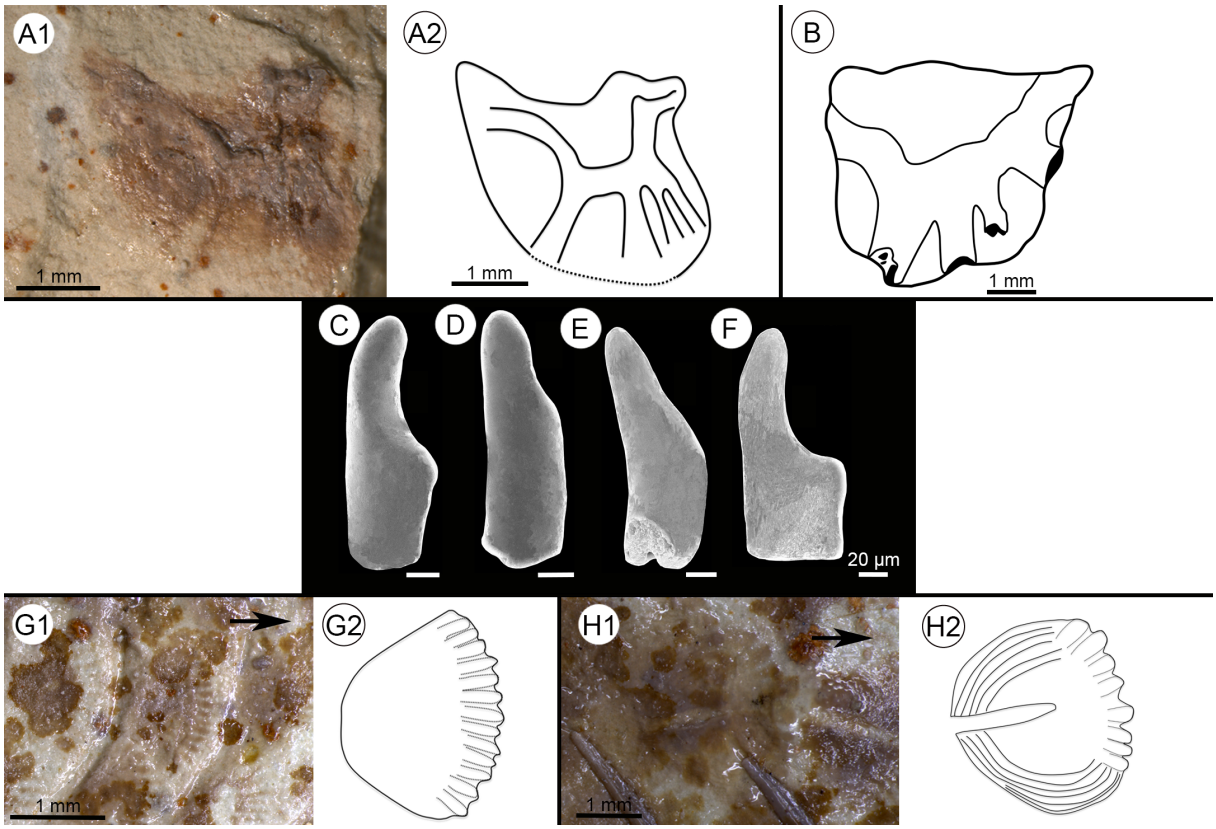


**Figure 3. A1–A2**, Lacrimal of †*Protochromis pickfordi* gen. et sp. nov. (OCO-5-22), showing the lateral line branched into six tubules; **B**, lacrimal of cf. *Pelmatochromis* spp. (redrawn after Van Couvering, 1982); **C–F**, SEM pictures of pharyngeal teeth of †*Protochromis pickfordi* gen. et sp. nov. (OCO-5-22); **C**, hook-like unicuspid tooth with shoulder; **D**, simple unicuspid tooth; **E**, simple unicuspid tooth; **F**, hook-like unicuspid tooth with shoulder; **G1–H2**, scales of †*Protochromis pickfordi* gen. et sp. nov.; **G1–G2**, flank scale with 13 radii (OCO-5-35); **H1–H2**, lateral line scale with tubular opening on peduncle; circuli are also discernible (mirror image; OCO-5-22). The arrows point anteriorly.

**Vertebral column.** The vertebral column contains 28 (15 + 13) vertebrae. The first two and the last two vertebrae are short. The neural spine of the first vertebra is not recognizable. The neural spines are short at the anterior end of the vertebral column, and become more elongated posteriorly, reaching their maximum length at the level of the end of the spinous part of the dorsal fin (vertebrae 6-20) before shortening again along the caudal peduncle. The haemal spines show a similar pattern, with long spines at the origin of the anal fin becoming progressively shorter towards the caudal fin (Fig. 1A1–A3). There are 13 pairs of robust ribs, which reach the margin of the abdominal cavity and are connected to the centra by strong parapophyses. The first pair of ribs originates on the third vertebra (Fig. 1A1–A3). Epineurals and supraneurals/predorsals are not discernible.

**Median fins and support structures.** The caudal skeleton is similar to that of other members of the Cichlidae. Five hypural plates (Hyp1–5) are recognizable and the diastema is almost closed. Hyp1+2 and Hyp4 are large and triangular, Hyp3 is comparatively thin and separated

from Hyp4 by a fine suture, Hyp4 presents a crest-like thickening on its dorsal outline. Hyp5 is long and slender and reaches uroneural 1 proximally; it is separated from Hyp4 by the stegural. A long and widened parhypural, almost reaching the terminal centrum, is present, while a hypurapophysis is not recognizable (Fig. 1B1–B2). Two epurals are visible. The one positioned between the neural spines of PU3 and PU2 is long and broad, while the other, located on top of uroneural 1 between the neural spine of PU2 and hypural plate 5, is very short. None of these aforementioned structures is fused with the stegural. Two preural vertebrae (PU2, PU3) contribute to the caudal endoskeleton. PU2 has a strongly reduced neural spine, while its haemal spine is autogenous, broad, elongate and displays a long, slender anterior process. The neural spine of PU3 is not reduced and its haemal spine is duplicated. The caudal fin is truncate to subtruncate and comprises 16 (8+8) segmented principal rays that are supported by epural 1, the hypural plates 1–5, the parhypural and the haemal spine of PU2 (Fig. 1B1–B2). Seven short, unbranched procurrent rays are present both dorsally and ventrally, and are supported by epural 1, the neural spine of PU3 and the haemal spine of PU2.

The dorsal fin consists of 13 spines and nine rays (Table 2), lengths of spines increase from the first to the last spine. Each spine and each ray (apart from the last ray) is supported by an elongate and thin pterygiophore, and each pterygiophore is associated with its individual interneural space (Fig. 1A1–A3). The first pterygiophore inserts into the interneural space of vertebrae 1 and 2, while the last pterygiophore associated with a spine inserts behind the neural spine of vertebra 13 (Fig. 1A1–A3). The pterygiophores related to the rays shorten gradually towards the caudal fin.

The anal fin consists of three spines, which increase in length from spine I to spine III, and nine branched rays. The first two spines share one pterygiophore, while the third spine and the branched rays are each supported by a single pterygiophore, except for the last ray. The two anteriormost pterygiophores insert into the interhaemal space of the last abdominal vertebra (vertebrae 14 and 15). All pterygiophores gradually shorten towards the caudal fin (Fig. 1A1–A3).

**Paired fins and support structures.** Imprints of nine pectoral fin rays are visible. The cleithrum is a robust slightly bent bone, with the upper part anteriorly expanded and the lower part partially covered by the subopercular (Fig. 1A1–A3). The supracleithrum is long, straight and dorsorostrally tapered (Fig. 1A1–A3). Above this bone the posttemporal is

visible; it is crushed, but seems to be widely forked with equal arms. A long and slender postcleithrum extends from the anterior expansion of the cleithrum downwards to the pectoral fin rays and partially overlaps with the basipterygium (Fig. 1A1–A3). The scapula seems to be rectangular and has a large foramen; a coracoid is visible as an imprint underneath the scapula and cleithrum. Four rectangular radials are present, the ventralmost being the largest and the dorsalmost the smallest.

The pelvic fins are set low, and each is comprised of a strong spine and five branched rays that do not reach the anal fin (Fig. 1A1–A3). It is not possible to decide whether one ray is more elongate than the others. The pelvic fin spine presents lateral and medial processes for articulation with the pelvic bone. The basipterygia are triangular in shape (Fig. 1A1–A3), the proximal tip is not recognizable.

**Squamation.** Cycloid scales are visible on the flanks and the peduncle (Figs. 1A1–A2, 2A1–B4, 3H1–H2), and are especially well preserved on the caudal peduncle along the lateral line (Figs. 1A1–A2, 2A1–B4). Some scales are present along the base of the soft-rayed part of the dorsal fin. Whether these scales are displaced or not cannot be determined with certainty. Scales carry approximately nine to 13 radii and are longer in the vertical axis (= scale width, 1.09–1.38 mm) than the horizontal (= scale length, 1.0–1.19 mm,) (Fig. 3G1–H2). It is estimated that around 30 scales are present in the longitudinal line.

The lateral line (= trunk canal) is divided into three segments:

(i) The anterior segment consists of at least 14 lateral-line scales, 11 of which have a tubular opening. Ten of the 14 lateral-line scales form a row that extends across the middle of the neural spines of vertebrae 11–18 (Figs. 1A1–A3, 2A1–A2, 2C). The first two scales in this row are tubular, and these are followed by two normal scales (without a sensory opening), one with a tubular opening, one normal scale and then again four scales, each with a tubular opening. The remaining lateral-line scales are dislocated. Two of them appear above the first two spines of the dorsal fin, and two are positioned above spines 9 and 10 of the dorsal fin.

(ii) Of the two posterior segments, one is positioned below the anterior trunk canal segment and separated from it by a vertical gap of two scale rows (Figs. 1A1–A3, 2A1–A2, 2C). It consists of 12 scales arranged in a row that extends across the middle of the haemal spines from vertebra 18 to the terminal centrum. The first three scales are pitted, the fourth is normal, and then follow three scales with a tubular opening, two normal scales, and again two scales with a tubular opening. The first pitted scale ‘overlaps’ with the last tubular scale of the

anterior trunk canal segment (with a gap of two scale rows in between), and also with the first pitted scale from the second posterior trunk canal segment (with a gap of one scale row in between; see below).

(iii) The second segment of the posterior trunk canal is positioned dorsally and posterior to the anterior segment and separated from it by a single scale row. It consists of 10 scales arranged in a row that projects just above the neural spines of the caudal vertebrae (from vertebra 18 to the terminal centrum). This row starts with three pitted scales, which are followed by seven scales with a tubular opening (Figs. 1A1–A3, 2B1–C).

## 3.4. Discussion

### 3.4.1. Systematic demarcation

The apomorphic characters of the family Cichlidae have been compiled by Carpenter and Niem (2001), Fujita (1990), Nelson (2006), Takahashi and Nakaya (2002) and Sebilia and Andreata (1991). Accordingly, †*Protochromis pickfordi* can be securely identified as a member of the Cichlidae, based on its possession of the following combination of characters: number of principal caudal fin rays (8+8), pelvic fin formula (I/5), caudal skeleton with five hypurals, two epurals and free first uroneural, PU2 without neural spine, but with neural arch and autogenous haemal spine, PU3 with non-autogenous haemal spine. Moreover, Cichlidae possess a divided lateral line that is characterized by an anterior and a posterior trunk canal segment, with the anterior one positioned dorsally to the posterior and separated from it by a vertical gap of at least two scale rows (Webb, 1990). A divided lateral line is also present in the fossil (see also below).

Previously suggested synapomorphies characterizing the different clades within the living Cichlidae are related to soft tissue, the pharyngeal apophysis, details of the epibranchial bones, microstructures of scale surfaces, squamation patterns, lateral-line foramina on the head, and other delicate structures (e.g. Regan, 1920, 1922; Greenwood, 1978; Lippitsch, 1990; Stiassny, 1991; Casciotta and Arratia, 1993; Lippitsch, 1995; Kullander, 1998, 2003). Apart from squamation patterns and scale characters, which have been identified in some fossil cichlids (Van Couvering, 1982; Murray, 2000) and are also detectable in our specimen, the aforementioned characters are usually not recognizable in fossil cichlids. This is the reason why no character-state matrix for phylogenetic analyses that is applicable to fossils has

yet been developed for the cichlid subfamilies and tribes. However, in light of its late Miocene age, †*P. pickfordi* most probably represents a member of the African cichlids (subfamily Pseudocrenilabrinae), because the split between African cichlids and the Malagasy, Indian and South American cichlids is thought to have occurred in the Eocene, at the latest (Murray, 2001a; Azuma et al., 2008; Friedman et al., 2013).

In the following we discuss characters relating to the lateral line, the lacrimal, osteology, squamation and meristic traits in order to infer the phylogenetic relationships of †*P. pickfordi* within the Pseudocrenilabrinae. One very striking feature of †*P. pickfordi* is the presence of a tripartite lateral line. Among Pseudocrenilabrinae, a three-fold division of the lateral line system is known exclusively from two genera of the Ectodini (a tribe endemic to Lake Tanganyika), i.e. *Xenotilapia* Boulenger 1899 and *Grammatotria* Boulenger 1899 (e. g. Pellegrin, 1904; Poll, 1986). Intra-Ectodini phylogenetic relationships remain poorly resolved in molecular phylogenies based on either mitochondrial or nuclear DNA (Koblmüller et al., 2004; Weiss et al., 2015). It thus remains uncertain whether or not the emergence of an additional (third) trunk canal segment occurred only once within the Ectodini. Moreover, there is a notable difference between †*P. pickfordi* and *Grammatotria*/*Xenotilapia* in the position of the additional (third) trunk canal segment. In the latter, it lies below the posterior trunk canal segment (Poll, 1986), whereas it is positioned above this segment and also above the anterior segment in †*P. pickfordi* (Figs. 1A1–A3, 2). As a result, it is not unambiguously clear whether the third trunk canal segment of the fossil taxon is homologous to the third segment seen in modern *Xenotilapia* and *Grammatotria*. However, based on the fact that, among all African cichlids, a third lateral line segment appears solely within the Ectodini, we assume that this character state of †*P. pickfordi* is indicative of a phylogenetic proximity to the modern Ectodini.

In the following, we consider not only Lake Tanganyika tribes, but also species of *Orthochromis*, as well as *H. vanheusdeni*, which have been pinpointed as potential contributors to the cichlid radiation in Lake Tanganyika (Meyer et al., 2015; Weiss et al., 2015). The character "lateral line on anteriormost infraorbital [lacrimal] branched into six tubules" sensu Takahashi (2003b:368), as seen in †*P. pickfordi*, is restricted to six tribes of the Lake Tanganyika cichlids, i.e. Bathybatini, Perissodini, Limnochromini, Ectodini, Lamprologini and Eretmodini (Takahashi, 2003a) (see Fig. 4). In addition, both the rounded trapezoid shape of the lacrimal and the arrangement of the tubules seen in †*P. pickfordi* is

reminiscent of Limnochromini (Fig. 4). Furthermore, comparison of meristic characters in †*P. pickfordi* with those of Lake Tanganyika tribes that bear six tubules on the lacrimal reveals a close similarity to Ectodini and Limnochromini. Only the number of vertebrae is slightly lower in the fossil (28 vs. 30–37 and 29–35, respectively) (Fig. 4). A close affinity between †*P. pickfordi* and the Ectodini and Limnochromini is additionally supported by the PCoA based on selected meristic and osteological characters from our comparative dataset comprising all Lake Tanganyika lineages, species of *Orthochromis*, and *Haplochromis vanheusdeni*. The PCoA plot positions †*P. pickfordi* within the 95% confidence ellipses of these two modern tribes (Fig. 5). However, as already mentioned in the Methods section, the sample of taxa used for PCoA is almost complete for the 'most ancient mouth-brooders' and for Eretmodini, but not for the Lamprologini. Hence future studies should try to include more species of the latter to clarify the systematic significance of these results. At all events, the PCoA strongly suggests that the character set found in †*P. pickfordi* is not similar to those of the members of the 'most ancient mouth-brooders', the Eretmodini and the Lamprologini. As a low number of vertebrae (about 24) is the ancestral condition within cichlids (Van Couvering, 1982), the relatively low number of vertebrae observed in †*P. pickfordi* may therefore relate to its ancestral state with respect to the modern Ectodini and Limnochromini.

On the other hand, both Ectodini and Limnochromini have ctenoid scales, whereas cycloid scales are present in †*P. pickfordi*. Cycloid scales occur in all non-haplotilapiine Pseudocrenilabrinae (Tylochromini, Hemichromines, Chromidotilapiines and Pelmatochromines), but also in several tribes of the Haplotilapiines (e.g. Tilapiini, Steatocranini, Pelmatolapiini, Boulengerochromini, Bathybatini, Trematocarini, Perissodini) (Lippitsch, 1995, 1998; Dunz and Schliewen, 2013) (see Fig. 4). Among the six Lake Tanganyika tribes discussed above, only Bathybatini and Perissodini possess cycloid scales (Poll, 1986; Lippitsch, 1998), and the latter is closely related to Ectodini (Weiss et al. 2015). In summary, and taking together all characters discussed above, †*P. pickfordi* presents a mosaic-like character set, exhibiting traits found in four modern Lake Tanganyika tribes (Ectodini, Limnochromini, Bathybatini and Perissodini).

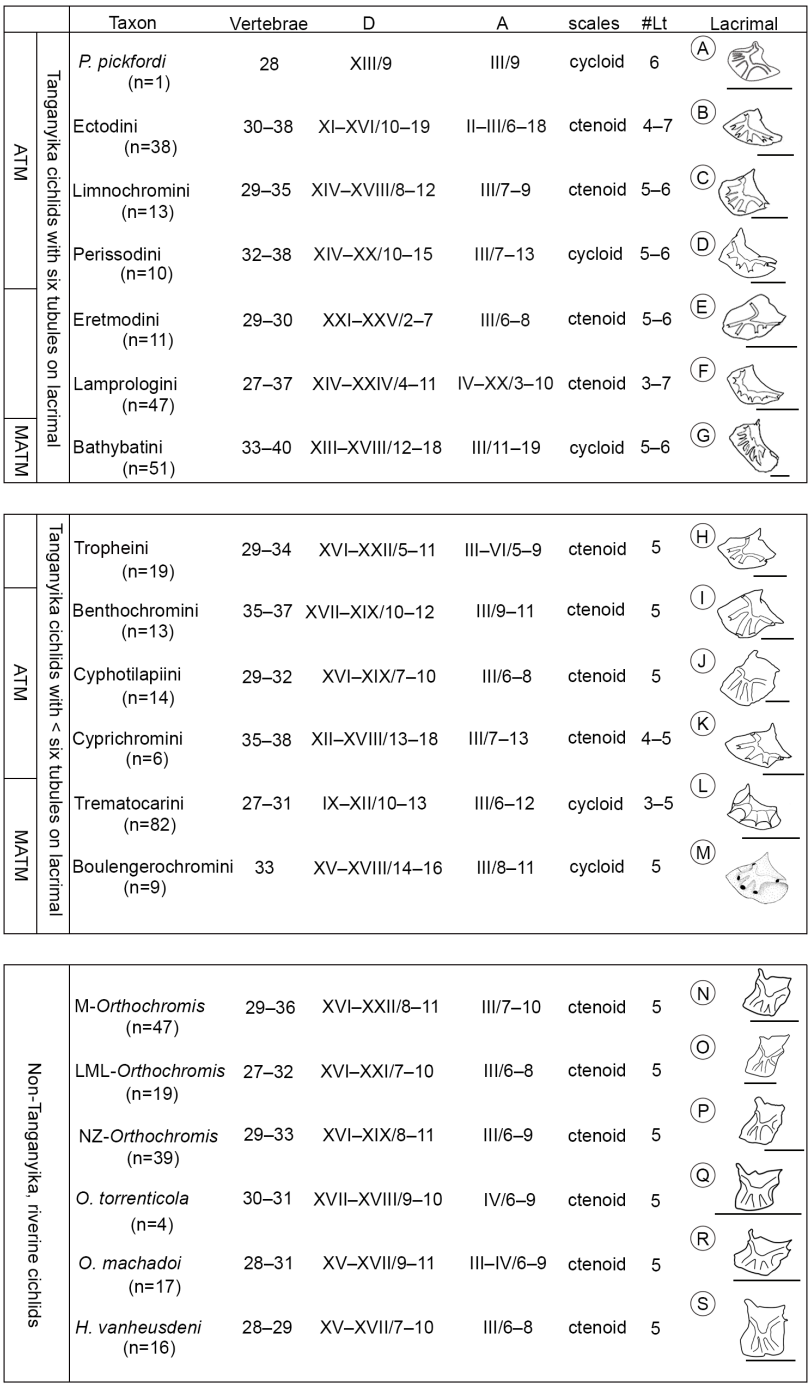


**Figure 4. Meristic counts, scale types and lacrimal morphology of †*Protochromis pickfordi* gen. et sp. nov. and representatives of all Tanganyika cichlid lineages plus members of the genus *Orthochromis* and the species *Haplochromis vanheusdeni*** (meristic counts from this study, Poll (1986) and De Vos and Seegers (1998); scale type from this study and Poll (1986); lacrimal morphology from this study (A, J, N–S), Liem (1978) (M) and Takahashi (2003a) (B–I, K–L)); species from which lacrimals are illustrated are abbreviated with letters (A, †*P. pickfordi*; B, *Ectodus descampsi;* C, *Greenwoodochromis christyi;* D, *Xenochromis hecqui*; E, *Eretmodus cyanostictus*; F, *Variabilichromis moorii*; G, *Bathybates minor*; H, *Lobochilotes labiatus*; I, *Benthochromis tricoti*; J, *Cyphotilapia* sp. (ZSM 043240_(P-AA-0999)); K, *Cyprichromis microlepidotus*; L, *Trematocara marginatum*; M, *Boulengerochromis microlepis*; N, *Orthochromis malagaraziensis* (ZSM 041469_(DRC-2011+1029)); O, *Orthochromis stormsi* (ZSM 042319_(P-AA-0708)); P, *Orthochromis* sp. Mambilima (ZSM uncatalogued specimen); Q, *Orthochromis torrenticola* (ZSM 038201_(Uli-LUB 2008+008)); R, *Orthochromis machadoi* (BMNH 1984.2.6.116-131_2); S, *Haplochromis vanheusdeni* (ZSM 043134). **Abbreviations**: **A**, anal fin formula; **ATM**, 'ancient Tanganyika mouth-brooders'; **D**, dorsal fin formula; **LML-*Orthochromis***, Luapula-Mweru system and Lualaba/Congo *Orthochromis*; **#Lt**, number of lateral line tubules on the lacrimal; **M-*Orthochromis***, Malagarasi *Orthochromis*; **MATM**, 'most ancient Tanganyika mouth-brooders'; **n**, number of specimens; **NZ-*Orthochromis***, Northern Zambian *Orthochromis*. All scale bars equal 5 mm.

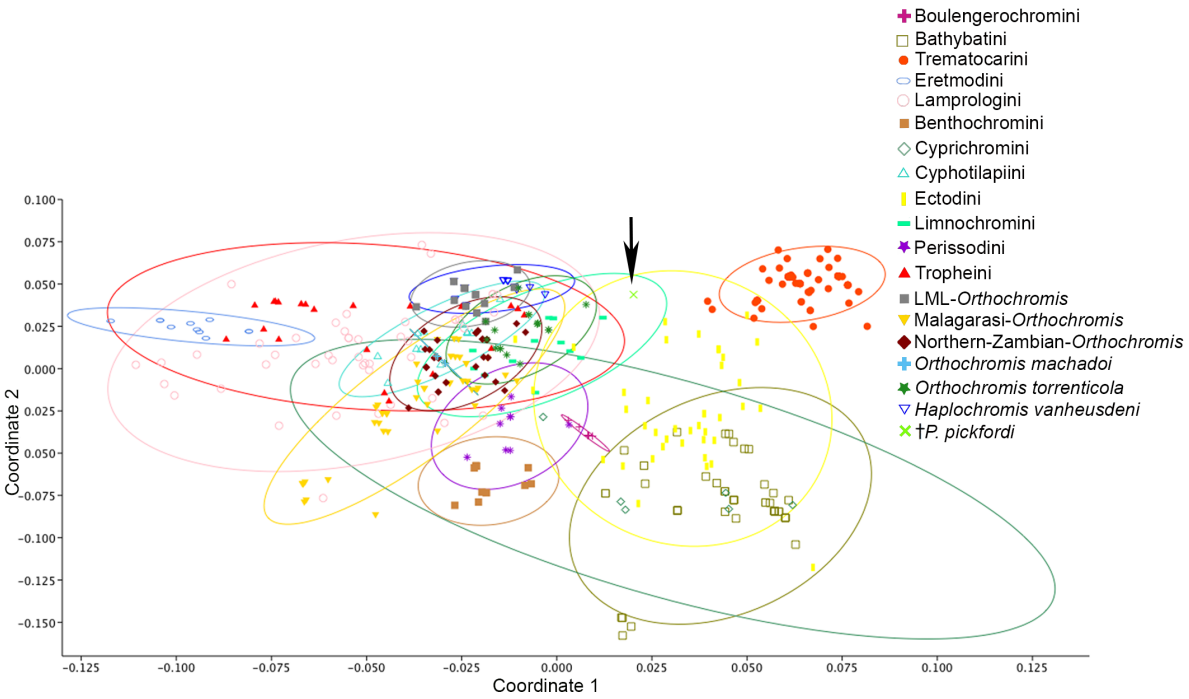


**Figure 5. Principal Coordinates Analysis (PCoA) scatter plot based on nine meristic and osteological characters of †*Protochromis pickfordi* gen. et sp. nov. (arrow) and the here examined Tanganyika cichlids plus species of *Orthochromis* and *Haplochromis vanheusdeni*** (N=456). Species score limits are visualized as 95% ellipses. Coordinate 1 vs. Coordinate 2. Coordinate 1 explains 58.77% and Coordinate 2 explains 31.08% of the variation.

### 3.4.2. Comparisons with previously described fossil cichlids

Most fossil cichlids from Africa and Arabia, and the single Pseudocrenilabrinae-like cichlid that has been found in Europe (*Oreochromis lorenzoi* Carnevale et al. 2003), can be separated from †*P. pickfordi* based on their meristic counts or osteological characters (see Table 3). In the following comparison we consider all previously described fossil cichlids from Africa, Saudi Arabia and Europe that display at least some of the characters which are preserved in †*P. pickfordi*. We have not used an elevated number of anal spines (more than 3) to discriminate between †*P. pickfordi* and other fossil cichlids here, because cichlids may show intraspecific variation for this character (see study of Trewavas, 1983 on several species of *Oreochromis*).

†*P. pickfordi* can be clearly differentiated from the fossil remains of cf. *Tylochromis* Regan 1920 described from Libya and Egypt by Otero et al. (2015) and Murray (2002, 2004), respectively, based on its unicuspid dentition (vs. molariform pharyngeal teeth in cf. *Tylochromis*). Moreover, counts of dorsal spines and vertebrae that differ from those for †*P. pickfordi* have so far been reported for †*Mahengechromis* spp. Murray 2000, unidentified

fossils referred to as 'Form B' Van Couvering 1982, †*Kalyptochromis hamulodentis* Van Couvering 1982, *Oreochromis lorenzoi* Carnevale et al. 2003, *Tilapia nigra* Trewavas 1937, and *T. crassispina* Arambourg 1947 (see Table 3 for details and references). Furthermore, *Oreochromis harrisae* Murray and Stewart 1999, *Tilapia fossilis* and *T. melanopleura* White 1937 possess a larger number of dorsal spines than that seen in †*P. pickfordi*, while their numbers of vertebrae are either unknown or slightly lower than the count for †*P. pickfordi*.

The remaining fossil cichlid species from North and East Africa (Van Couvering, 1982) and from Saudi Arabia (Weiler, 1970; Lippitsch and Micklich, 1998) share some characters with †*P. pickfordi*. Low numbers of dorsal fin spines comparable to that seen in †*P. pickfordi* have been reported for †*Macfadyena dabanensis* Van Couvering 1982, unidentified cichlids termed 'Form C' and 'Form D' (see Van Couvering, 1982), †*Nderechromis cichloides*, †*Palaeofulu kuluensis* Van Couvering 1982*,* †*Palaeochromis rouselleti* Sauvage 1907, †*P. darestei* Sauvage 1907, ?*Heterochromis* sensu Lippitsch and Micklich 1998 and undetermined cichlids described by Weiler (1970). Among these species, †*M. dabanensis*, 'Form C', †*P. kuluensis* and ?*Heterochromis* most probably have two predorsal bones (Van Couvering, 1982; Lippitsch and Micklich, 1998) and are therefore unlikely to be closely related to the Lake Tanganyika tribes (to which †*P. pickfordi* can be assigned, see above), which generally have only one predorsal (this study). Moreover, ?*Heterochromis* sensu Lippitsch and Micklich 1998 shows ctenoid rather than cycloid scales. 'Form D' can be separated from †*P. pickfordi* by its low number of vertebrae. †*N. cichloides* has more soft rays in the median fins than †*P. pickfordi* (A 10-12 vs. 9 and D 13 vs. 9) and its scales are ctenoid (Van Couvering, 1982) (vs. cycloid in †*P. pickfordi*). In †*Palaeochromis rouselleti* and †*P. darestei* the scale type and pharyngeal dentition is similar to †*P. pickfordi,* but the numbers of vertebrae are different (25-26 vs. 28 in †*P. pickfordi*) and the body of †*P. pickfordi* is more elongate (see Sauvage, 1910). The undetermined cichlids described by Weiler (1970) can also be separated from †*P. pickfordi* based on their lower number of vertebrae (23 vs. 28).

It is more difficult to conclusively distinguish between †*P. pickfordi* and several fossil cichlids described in open nomenclature, because their lateral line and meristic counts are not known. Among these latter forms are 'Tilapiini Group 2' and 'Tilapiini Group 3' from the Oligocene of Saudi Arabia (Lippitsch and Micklich, 1998) (Table 3). Given their much older age (Oligocene) and their geographical origin (Saudi Arabia), it is unlikely that †*P. pickfordi* represents the same taxon as either 'Tilapiini Group 2' or 'Tilapiini Group 3'. In addition,

'Tilapiini Group 3' has ctenoid scales, whereas the scales of †*P. pickfordi* are exclusively cycloid. In 'Tilapiini Group 2', Lippitsch and Micklich (1998) observed scales on the soft-rayed part of the dorsal fin. †*P. pickfordi* may also possess a row of scales on the soft-rayed part of the dorsal fin, but these scales could also be dislocated in our specimen.

Further cichlid fossils in open nomenclature have been described by Van Couvering (1982). These include 'Form A' from the Oligocene in Somalia and several Miocene taxa from Kenya, i.e. '?*Tilapia* Cichlidae Form A', 'Cichlidae Form C', 'Cichlidae Form D', 'Cichlidae spp. Group A' and 'Cichlidae spp. Group B' (see Table 3 for details). Given the Oligocene age of 'Form A', this taxon is certainly different from †*P. pickfordi*. However, of the Miocene taxa from Kenya only 'Cichlidae Form D' can be clearly differentiated from †*P. pickfordi*, because it possesses molariform pharyngeal teeth (vs. unicuspid in †*P. pickfordi*). Similarly, the pharyngeal teeth of 'Cichlidae spp. Group A' and 'Cichlidae Form C' differ from those of †*P. pickfordi*. The remaining taxa, all of which are incompletely preserved, share with †*P. pickfordi* the pharyngeal dentition and/or the cycloid scales, and could possibly belong to †*P. pickfordi*.

Two further species deserve special consideration. The first is †*Oreochromis* (*Sarotherodon*) *martyni* (Van Couvering, 1982) which, like †*P. pickfordi*, was recovered from the Ngorora Formation of the Tugen Hills, albeit in a slightly older stratigraphical context (middle Miocene). The meristic counts for both are rather similar, but *O.* (*Sarotherodon*) *martyni* has a divided lateral line of the general cichlid type (see Van Couvering, 1982) and can therefore be definitively discriminated from †*P. pickfordi*. The second taxon of interest here is cf. *Pelmatochromis* spp. Van Couvering 1982, found in the lower Miocene of Uganda. This fossil cichlid has been described on the basis of disarticulated bones, hence no meristic counts are known. However, it features a lacrimal with the same overall shape and six foramina as seen in †*P. pickfordi* (see Table 3 and Figs. 4 and 9) and both forms have cycloid scales. In her study, Van Couvering (1982) compared cf. *Pelmatochromis* with four species previously identified as *Pelmatochromis*, and noted that her fossil displays a mixture of "primitive and derived" (Van Couvering, 1982:40) characters, such as the morphology of the pharyngeal apophysis, the ascending process of the premaxilla, the pars jugularis and the hyomandibula, and the presence of leaf-shaped unicuspid pharyngeal teeth, weakly bicuspid pharyngeal teeth and cycloid scales. In the meantime, three of the four extant species that Van Couvering (1982) had used for comparison have been reassigned to different chromidotilapiine genera,

i.e. *Chromidotilapia* and *Pelvicachromis*. Moreover, all present-day Pelmatochromines and Chromidotilapiines show only four or five tubules on the lacrimal (Trewavas, 1983; Stewart and Roberts, 1984; Greenwood, 1987; Lamboj, 2000, 2004, 2005; Schliewen and Stiassny, 2006; Stiassny and Schliewen, 2007; Lamboj, 2014; Lamboj et al., 2014). Therefore, it is unlikely that cf. *Pelmatochromis* spp. sensu Van Couvering (1982) is a member of the Pelmatochromini or Chromidotilapiini and it could, like †*P. pickfordi*, represent a precursor lineage of the Lake Tanganyika tribes instead.

**Table 3. Summary table of relevant characters of the fossil specimen and all previously described African, Saudi Arabian, and European fossil cichlids, where at least some of these characters are preserved**. Extinct species are marked with †. **Abbreviations**: **A**, anal fin formula; **D**, dorsal fin formula; **LL**, number of lateral line segments; **$L_r$**, scales in longitudinal row; **#$L_t$**, number of tubules on the lacrimal; **Pd**, number of predorsals; **Pha.**, pharyngeal teeth; **Scales**, scale type; **V**, number of vertebrae; /, or. Fossil taxa are arranged in accordance to the author.

| Taxon | Reference | Age | Locality | Pd | V | D | A | Scales | Pha. | LL | $L_r$ | #$L_t$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| †*Protochromis pickfordi* | This study | Upper Miocene | Waril, Kenya | 1 | 28 | XIII/9 | III/9 | Cycloid | Unicuspid | 3 | 30? | 6 |
| †*Mahengechromis* spp. | Murray (2000) | Eocene | Mahenge, Tanzania | 1 | 22–25 | XV/8–9 | III/8–10 | Ctenoid | Unicuspid | 2 | 30 | 4–5 |
| cf. *Tylochromis* | Murray (2002) | Upper Eocene | Jebel Qatrani Fm., Egypt | – | – | – | – | – | Molariform | – | – | – |
| cf.? *Tylochromis* | Otero et al. (2015) | Upper Eocene | Dur At-Talah, Libya | – | – | – | – | – | Molariform | – | – | – |
| †*Macfadyena dabanensis* | Van Couvering (1982) | Oligo-cene | Daban Beds, Somalia | 2 | 27 | XIV/9 | III/10? | – | Bicuspid | 1+ | – | – |
| Form A | | | | – | – | – | – | Cycloid | Uni-, bi-, tric. | – | – | – |
| Form B | | | | – | – | XI/13 | III?/10 | Cycloid | Uni- & bic. | 2 | 22 | – |
| Form C | | | | 2? | – | XIV/8+ | ? | Ctenoid | – | – | – | – |
| Form D | | | | – | 24 | XIII/11 | VI/12 | ? cycloid | – | – | – | – |
| †*Kalyptochromis hamulodentis* | | Lower Miocene | Kulu Fm., Kenya | 2 | 30 | XVII/10 | IV/12 | Cycloid | Unicuspid | – | – | – |
| †*Nderechromis cichloides* | | | | – | – | XIV/13 | III/10–12 | Ctenoid | Bicuspid | – | – | – |
| †*Palaeofulu kuluensis* | | | | 2 | 25–29 | XII–XVI/7–13 | III/7–10 | Cycloid | Bicuspid | 2 | – | 4–5 |
| *?Tilapia,* Cichlidae Form A | | | Kulu Fm., Kenya | – | – | – | – | Cycloid | – | – | – | – |
| *Pelmatochromis* sp. | | | Lamitina Beds, Uganda | – | – | – | – | Cycloid | Bicuspid | – | – | 6 |
| Cichlidae Form C | | Early Miocene | Turkana Grits, Kenya | – | – | – | – | Cycloid | bicuspid | – | – | – |
| Cichlidae Form D | | Early Miocene | Turkana Grits, Kenya | – | – | – | – | Cycloid | molariform | – | – | – |
| †*Oreochromis martyni* | | Middle – upper Miocene | Ngorora Fm., Kenya | 1 | 27–29 | XIII–XIV/9–11 | III/7–11 | Cycloid? | Uni & bic. | 2 | – | – |
| Cichlidae spp. Group A | | Middle Miocene | Kirimun Beds, Kenya | – | – | – | – | – | bicuspid or round & flat | – | – | – |

**Table 3. (Continued)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cichlidae spp. Group B | | | | – | – | – | – | Cycloid | – | – | – | – |
| †*Palaeochromis rouselleti* | | Upper Miocene | Seybouse Valley, Algeria | 1 | 25–26 | XIII–XIV/9–14 | III/8 | Cycloid | Uni & bic. | – | – | – |
| †*Palaeochromis darestei* | | | | 1 | 25–26 | XII–XIV/10–16 | III/9–10 | Cycloid | Uni & bic. | 2 | – | – |
| ?*Heterochromis* | Lippitsch & Micklich (1998) | Oligo-cene | Ad Darb, Baid Fm., Saudi Arabia | 1+ | – | XIII–XIV/13+ | IV–V/– | Ctenoid | – | – | – | – |
| Tilapiini Group2 | | | | – | – | – | III?V/– | Cycloid | – | – | – | – |
| Tilapiini Group3 | | | | – | – | – | IV/– | Ctenoid | – | – | – | – |
| Cichlidae indet. | Weiler (1970) | Oligo-cene – Miocene | Wadi Araba, Jordan | – | 23 | XII/5–9? | III/8 | Cycloid | – | – | – | – |
| †*Oreochromis lorenzoi* | Carneval e et al. (2003) | Upper Miocene | Gessoso-Solfifera Fm., Italy | 1 | 30 | XV/12 | IV/8 | Cycloid | Unic., kukri | 2 | 26? | 5 |
| †*Oreochromis harrisae* | Murray & Stewart (1999) | Lower Pliocene | Middle Awash, Ethiopia | 1 | – | XVI?/11? | – | Cycloid | Unicuspid | 2 | 31 | 5 |
| †*Tilapia fossilis* | White (1937) | Upper Pleisto-cene | Ashanti, Lake Bosumtwi, Kenya | – | 27 | XV/12 | III/7–8 | Cycloid | Unicuspid | 2 | – | – |
| †*Tilapia melanopleura* | | | | – | – | XV?/– | III/12? | Cycloid | Uni- & bic. | 2 | – | – |
| †*Tilapia nigra* | Trewava s (1937) | Lower Pleisto-cene | Kavirondo Province, Kenya | – | 30 | XVII–XVIII/10–12 | IV–V/9–11 | – | – | – | 30–32 | – |
| †*Tilapia crassispina* | Arambo urg (1947) | Lower Pleisto-cene | Omo Valley, Kenya | – | 30 | XVI/– | III/8 | Cycloid | Bicuspid | – | 35? | – |

### 3.4.3. Evolutionary history of the Lake Tanganyika cichlids

The temporal relationship between cichlid diversification in the Rift Valley and the colonization of Lake Tanganyika is the subject of controversial and ongoing discussion. Problems in interpreting the evolutionary history of the cichlids of Lake Tanganyika have arisen from discordances between phylogenetic trees constructed on the basis of mitochondrial *vs.* nuclear markers, incomplete taxon sampling, uncertainty with respect to the geological age of Lake Tanganyika itself, and a scarcity of cichlid fossils from Africa with unambiguous phylogenetic placement (see Meyer et al., 2015; Weiss et al., 2015). Estimates of the geological age for the Lake Tanganyika Basin range from 5.5 to 14.5 Ma, depending on the methods used. Cohen et al. (1993) extrapolated Pleistocene sedimentation rates to the Miocene and concluded that the Lake Tanganyika Basin formed 9–12 Ma. A slightly older age (14.5 Ma) was proposed on the basis of combined litho- and biostratigraphic data (Roller et al. 2010), whereas a drastically younger age (5.5 Ma) has been suggested based on thermochronology (Spiegel et al., 2007; Bauer et al., 2010). Previous studies dealing with molecular data and cichlid diversification have generally accepted the age suggested by Cohen et al. (1993), which has led to the widespread assumption that the cichlids of Lake Tanganyika must have originated less than 12 Ma (Salzburger et al., 2005; Day et al., 2008; Sturmbauer et al., 2010; Koblmüller et al., 2012).

In a recent study, Weiss et al. (2015) presented both mitochondrial and nuclear DNA data based on a comprehensive sample of taxa including all Lake Tanganyika cichlid lineages (sensu Poll, 1986; Nishida, 1991; Meyer, 1993), as well as potential precursor lineages. Their analyses suggested that some Tanganyika lineages exhibit a mosaic genomic structure, most probably due to repeated hybridization, introgression and gene flow from riverine cichlids and from other Tanganyika lineages (see also Clabaut, 2005; Meyer et al., 2015). Furthermore, Weiss et al. (2015) recognized four major groups of the Lake Tanganyika cichlid fauna, which appear to have originated from four different founder lineages. These groups are: (i) the ‘most ancient Tanganyika tribes’, (ii) Lamprologini and Eretmodini, (iii) the ‘ancient Tanganyika mouth-brooders’, and (iv) Tropheini. Our study shows †*P. pickfordi* to be morphologically intermediate between the Limnochromini and Ectodini (see above), both of which belong to the ‘ancient Tanganyika mouth-brooders’. The presence of cycloid scales (vs. ctenoid scales in Limnochromini and Ectodini) shows that †*P. pickfordi* shares a character state with other ‘ancient Tanganyika mouth-brooders’ (e.g. Perissodini) and perhaps even with members of the ‘most ancient Tanganyika’ tribes. Therefore, we suggest that †*P.*

*pickfordi* may represent a lineage closely related to the precursor lineage that gave rise to the modern tribes of the 'ancient Tanganyika mouth-brooders'.

As Lake Tanganyika is located in the western branch of the EARS, the discovery of a potential Tanganyika-cichlid precursor from the late Miocene in the eastern branch of the EARS (Central Kenya Rift) supports the 'melting-pot Tanganyika hypothesis' (Weiss et al. 2015). This hypothesis suggests that precursor lineages of the modern Lake Tanganyika cichlids originated in rivers and wetlands prior to the formation of the lake, and possibly in areas beyond the boundaries of the present-day Lake Tanganyika drainage. In other words, the primordial Lake Tanganyika was initially colonized by an already diversified cichlid fauna.

The geological age of †*P. pickfordi* may serve in future studies as a new minimum age for the lineage that gave rise to the 'ancient Tanganyika mouth-brooders'. Molecular clock analyses have resulted in highly variable node-age estimates for the origin of Lake Tanganyika cichlid lineages, depending on the calibration point used (cichlid fossils, break-up of Gondwana or formation of the Tanganyika Basin). These dates range from younger than 12 Ma (formation of the Lake Tanganyika Basin; e.g. Koblmüller et al., 2012) to 20–26 Ma (fossil; Schwarzer et al., 2009) to 22–51 Ma (Gondwana; Genner et al., 2007). Our analysis of †*P. pickfordi* argues that the split between the 'most ancient Tanganyika mouth-brooders' and the Lamprologini/Eretmodini lineages must date back to at least 9 million years, but that the 'ancient Tanganyika mouth-brooders' had not necessarily radiated within the lake at that time. Furthermore, the find spot of †*P. pickfordi* and the fossil's inferred phylogenetic relationships support the existence of an ancient hydrological connection (e. g. via a proto-Malagarasi River) between the Central Kenya Rift and Lake Tanganyika, as proposed in previous geological studies (Coulter, 1991; Cohen et al., 1997; Goodier et al., 2011). This connection was probably disrupted in the initial stages of rifting - close to, or coincident with the 'Nyanja event', i.e. the initial flooding of the Lake Tanganyika Basin (Rosendahl, 1988) which, according to Cohen et al. (1993), occurred around 9-12 Ma (see also Lezzar et al., 1996; Cohen et al., 1997). This scenario would not be in conflict with our data, because the lake sediments in which †*P. pickfordi* was found are 9-10 Myr old. It should also be noted in this context that a Miocene Trans-African East-West directed hydrological network has previously been suggested for the Upper Nile and the Chad Basin (Otero et al., 2009).

## 3.5. Conclusion

Based on meristic and osteological data derived from all present-day Lake Tanganyika cichlids plus *Orthochromis* (tribe Haplochromini) and *Haplochromis vanheusdeni*, we propose that the newly discovered cichlid fossil from the upper Miocene of Central Kenya is most likely related to the precursor lineage that gave rise to the modern tribes of the 'ancient Tanganyika mouth-brooders'. This result implies that the use of a comprehensive set of comparative material derived from extant cichlids may make it possible to phylogenetically place other fossil cichlids with greater confidence in future studies.

Apart from a lower Miocene cichlid from Uganda ('cf. *Pelmatochromis* spp.'), none of the previously described fossil cichlid taxa from Africa, Arabia and Europe possess distinctive similarities to †*P. pickfordi*. This indicates that the Ngorora fish Lagerstätte in Central Kenya may provide an unrivaled window into the evolutionary history of African cichlids, particularly into the 'East African Radiation', which has led to the megadiversity of the present-day cichlids in Lake Tanganyika, Lake Malawi and Lake Victoria.

Because †*P. pickfordi* shares derived character states with at least two tribes of the 'ancient Tanganyika mouth-brooders', the origin of the stem lineage of the 'ancient Tanganyika mouth-brooders' must predate the age of the †*P. pickfordi* fossil. It can therefore be assumed that the new fossil cichlid sets the minimum age for that stem lineage at 9 Myr.

Furthermore, the new fossil provides additional support for the presence of an ancient East-West connection (e. g. proto-Malagarasi River) between the Central Kenya Rift and Lake Tanganyika, which is consistent with previous assumptions regarding the hydrological networks across East and Central Africa during the Miocene.

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# 4. Exceptionally well preserved fossil cichlids from the Miocene Ngorora Fish Lagerstätte of Central Kenya with the description of †*Baringochromis* gen. nov.

Melanie Altner, [1] and Bettina Reichenbacher [1]
[1]Department of Earth and Environmental Sciences, Paleontology & Geobiology, Ludwig-Maximilians-Universität München, Munich, Germany

**Abstract**

Cichlids represent one of the most species-rich groups of tropical freshwater fishes, and display remarkably high levels of diversity in East Africa. Fossil cichlids from the region offer unique insights into the evolutionary history of these radiations. Here we describe †*Baringochromis* gen. nov., a fossil cichlid taxon from the upper Miocene (9–10 Ma) of the East African Rift System, based on a total of 335 well-preserved specimens recently recovered from the Ngorora fish Lagerstätte in Central Kenya. †*Baringochromis* gen. nov. displays a unique combination of characters, with one predorsal bone, six infraorbitals including a lacrimal with four or five lateral line tubules, a suboperculum partially covered with scales, cycloid scales on the body and head, 26–30 vertebrae, and oligacanthous dorsal and anal fins. Moreover, based on differences in head and body shape and oral-tooth morphology, four species of †*Baringochromis* gen. nov. can be distinguished in our assemblage, which are interpreted as a species flock. Comparative analysis based on a comprehensive dataset of meristic, osteological and otolith data for extant haplotilapiine cichlids indicates that †*Baringochromis* gen. nov. occupies an intermediate position between the virtually pan-African Oreochromini and the East African Haplochromini. This conclusion is further supported by a Principal Coordinates Analysis (PCoA) based on meristic data. Previous molecular studies have revealed that riverine forms played a major role in the evolution of lacustrine cichlid diversification. Following the recent discovery of †*Protochromis pickfordi* from the same Lagerstätte, †*Baringochromis* gen. nov. is the second fossil taxon that strongly supports this scenario.

## 4.1. Introduction

With more than 220 genera and 1400 species, the family Cichlidae is one of the most diverse families of freshwater fish known today (Kolm et al., 2006; Fitzsimmons and Watanabe, 2010). They are distributed in the tropics and subtropics, with especially striking hotspots of diversity in the lakes of East Africa (e.g. Skelton, 2001; Salzburger and Meyer, 2004; Chakrabarty, 2006). Phylogenetic relationships within the family are well understood, based on both morphological and molecular data (e.g. Sparks and Smith 2004). All African cichlids are assigned to the subfamily Pseudocrenilabrinae, within which six major lineages can be distinguished: the Heterochromini, Tylochromini, Chromidotilapiines, Hemichromines, Pelmatochromis and Haplotilapiines (Schwarzer et al., 2009; Dunz and Schliewen, 2013). The most speciose of these major clades is Haplotilapiines, which itself comprises the lineages Etiini, Oreochromini, Boreotilapiines (Coelotilapiini, Heterotilapiini, Coptodonini, Gobiocichlini), Austrotilapiines ('East African Radiation', Tilapiini, Steatocranini), and Pelmatotilapiini (Schwarzer et al., 2009; Dunz and Schliewen, 2013). The 'East African Radiation' (EAR) is particularly rich in species because it includes the great radiations in Lakes Tanganyika, Malawi and Victoria (e. g. Meyer et al., 1990; Sturmbauer and Meyer, 1993; Moran et al., 1994; Sturmbauer et al., 1994; Loh et al., 2013).

Even though recent cichlids are commonly used as model organisms in evolutionary studies, their own evolutionary history has yet to be fully explored. This is in part due to the paucity of their fossil record, which currently comprises 18 articulated fossil cichlid taxa from Eocene to Pliocene sediments of Africa and Arabia, including the new cichlid †*Protochromis* (see Altner et al., (submitted) for details), together with isolated bones, scales and teeth (Stewart 2001). The objective of this study is to present a taxonomic analysis of newly discovered fossil cichlids from the Ngorora Formation (Central Kenya, East African Rift system) based on a comprehensive comparative dataset comprising meristic, osteological and otolith data from present-day haplotilapiine cichlids.

### 4.1.1. Geological setting

**Study site.** The fossil material described here was collected at the locality Waril (0°40′56.21′′N 35°43′7.43′′E) in the Kerio Valley, which lies to the west of the Tugen Hills in the Central Kenya Rift Valley (see Rasmussen et al., 2015). The tuffaceous lacustrine siltstones containing the fish fossils (Pickford et al., 2009) form part of the Ngorora fish Lagerstätte, and are of upper Miocene age (9–10 Ma). According to previous studies, the

palaeolake Waril was comparatively deep and alkaline, and the climate at that time was seasonally dry (Pickford, 1978; Jacobs, 2002; Kingston et al., 2002; Tiercelin and Lezzar, 2002; Bonnefille, 2010; Bamford et al., 2013; Rasmussen et al., 2015). The site has already yielded the fossil cichlid taxon *Protochromis pickfordi*, which has been interpreted as an ancient member of a lineage that contributed to the cichlid fauna of Lake Tanganyika (Altner et al., submitted).

## 4.2. Materials and Methods

### 4.2.1 Fossil material

The material consists of 335 specimens, of which 52 are complete (i.e. their standard lengths (SL) could be measured; total lengths (TL) were also determined for 41 of these). They were collected in 2013 and 2014 from the lacustrine sediments of the Ngorora Formation in the Tugen Hills. The material has been deposited in the Museum in Kipsaraman, Kenya, which is affiliated with the National Museum of Kenya in Nairobi.

### 4.2.2. Comparative material

A comparative x-ray dataset was assembled based on specimens from the State Zoological Collection in Munich and the Natural History Museum in London. It comprises a total of 297 species (125 genera, 22 lineages, 1171 specimens) representing all present-day haplotilapiine lineages and genera (according to Schwarzer et al., 2009; Dunz and Schliewen, 2013; Weiss et al., 2015), with the exception of *Baileychromis* Poll 1986 and *Pseudosimochromis* Nelissen 1977, which were not available.

Moreover, a comparative otolith dataset has been compiled based on specimens from the Museum of Natural History in Vienna and the State Zoological Collection in Munich, and on material described in published work (Gaemers, 1984, 1986; Tichy and Seegers, 1999; Artzi et al., 2009) and in the AFORO database (Lombarte et al., 2006). It comprises otoliths from 62 specimens (42 species, 29 genera and 22 lineages) representing all contemporary haplotilapiine lineages that fall within the definition mentioned above.

**Note.** We follow previous authors in using the suffix '-ines' for informal group names and reserving 'ini' for the formal names of tribes (see Schwarzer, 2011).

### 4.2.3. Morphological analyses

During fossil preparation, obscuring sediment was carefully removed under the microscope. Anatomical illustrations were made based on digital images taken with a digital camera mounted on a stereomicroscope. SEM images of teeth and otoliths were taken on a LEO 143VP at 15 kV and picture quality was enhanced using Photoshop CS6. Radiographs were produced using a Faxitron UltraFocus LLC x-ray unit. Morphometric measurements and meristic counts were compiled according to Holčík (1989) and Barel et al. (1977). Measurements were obtained from digital images in ImageJ version 1.49v (Rasband, 1997–2015) and recorded to the nearest 0.01 mm. Dorsal and anal fin-ray counts included every discernible ray, regardless of whether or not it was associated with a pterygiophore, and counts of vertebrae exclude the terminal centrum. Measurements on the fossils were standardized based on the body length (BL), i.e. the distance from the posterior margin of the operculum to the posterior margin of the hypural plate. If specimens were preserved as part and counterpart, both parts were included in the osteological and meristic analysis, while only the better preserved part was measured. Interpretation of osteological characters follows Van Couvering (1982), Trewavas (1983), Poll (1986), Kullander (1998), Lippitsch (1995, 1998) and Takahashi (2003b, 2003a).

### 4.2.4. Statistical analyses

A Principal Coordinates Analysis (PCoA) as implemented in the program PAST 3.11 (Hammer et al., 2001) was conducted on the basis of the characters noted in the fossil and inspection of the corresponding characters in the comparative dataset (see above). Characters used as variables included counts of spines and rays in the dorsal and anal fins, counts of abdominal, caudal and total vertebrae, the number of predorsals and the position of the vertebra associated with the pterygiophore of the last dorsal fin spine. The PCoA calculates a distance matrix in which the distance between objects reflects their degree of dissimilarity in Euclidean space (Leyer and Wesche, 2007). Unlike Principal Components Analysis (PCA), PCoA is not based on the correlation or covariance coefficient, but can make use of any measure of association (Zuur et al., 2007).

**Institutional abbreviations.** AMNH, American Museum of Natural History; BMNH, Natural History Museum, London; CU, Cornell University Museum of Vertebrates; IRSNB, Institut Royal des Sciences Naturelles de Belgique, Bruxelles; MNHN, Musée national d' histoire naturelle, Paris; MRAC, Royal Museum for Central Africa, Tervuren; NMW, Naturhistorisches Museum, Wien; NRM, Naturhistoriska Riksmuseet, Stockholm; RG, Royal

Museum for Central Africa; ROM, Royal Ontario Museum, Toronto; SAIAB, South African Institute for Aquatic Biodiversity, Grahamstown; SMF, Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt; ZSM, Bavarian State Collection of Zoology, Munich.

## 4.3. Systematic Paleontology

CICHLIDAE Bonaparte, 1835

PSEUDOCRENILABRINAE Fowler, 1935

HAPLOTILAPIINES Schliewen and Stiassny, 2003

†*Baringochromis* gen. nov.

(Figs. 1–14)

**Type species.** †*Baringochromis senutae* sp. nov.

**Included species.** †*Baringochromis sonyii*, †*Baringochromis stellae*, †*Baringochromis davidae* spp. nov.

**Etymology.** From Baringo, the name of the county in which the fossils were found, and *chromis* (Greek), a noun often used to refer to cichlids.

**Generic Diagnosis.** †*Baringochromis* can be distinguished from other cichlids by the following combination of characters: single predorsal bone; six infraorbitals, the first of which - the lacrimal - shows the lateral line branched into four or five tubules; tricuspid and/or unicuspid oral dentition; suboperculum partially scaled; cycloid scales on body and head; low numbers of fin spines and rays (D XI–XIII/6–10; A III/6–10); 26–30 vertebrae.

### 4.3.1. DESCRIPTION

**General description.** Measurements and meristics of †*Baringochromis* gen. nov. are given in Table 1. †*Baringochromis* is a low-bodied cichlid (Figs. 1–4) reaching 14.0–81.0 mm in standard length and 22.0–91.0 mm in total length. Most of the specimens are preserved in lateral view (with the head in lateral or dorso-lateral view), indicating that these fish are fairly narrow in body width compared to depth. The point of maximum body depth (41.0 ± 5.4% of BL) is located between the head and the origin of the pelvic fins. The minimum body depth (15.0 ± 2.5% of BL) is found on the posterior part of the caudal peduncle, close to the hypural plates. The depth of the head (45.4 ± 6.3% of BL) is equal to or slightly exceeds the greatest body depth. The dorsal profile of the head varies between species. The mouth is terminal but

slightly prognathous, with the lower jaw being longer (39.3 ± 5.1% of HL) than the upper (27.0 ± 4.2% of HL). The dorsal profile of the body is nearly straight from the supraoccipital crest to the end of the dorsal fin, and straight to slightly concave from the end of the dorsal fin to the caudal fin. The ventral profile of the body is gently curved from the lower jaw to the onset of the caudal peduncle, and straight to slightly concave along the caudal peduncle (Figs. 1–4). The caudal peduncle is moderately long (31.5 ± 4.1% of BL).

**Table 1. Morphometric measurements and meristic counts of the specimens of †*Baringochromis* gen. et sp. nov.**

| Character | Mean ± SD (Range) mm | Mean ± SD (Range) %BL |
|---|---|---|
| TL (41) | 64.4± 15.8 (22.4–90.9) | 174.4± 11.8 (137.7–195.6) |
| SL (52) | 52.9±16.7 (13.8–80.8) | 150.0 ± 9.4 (107.7–168.7) |
| BL (65) | 36.2±9.7 (10.6–55.4) | – |
| HL (52) | 18.0±5.7 (3.5–28.2) | 50.4 ± 7.4 (32.8–69.1) |
| HH (38) | 16.9±4.9 (6.0–25.6) | 45.4 ± 6.3 (31.8–59.4) |
| Orbit %HL (14) | 24.4±7.1 (14.9–43.8) | 24.4 ± 7.1 (14.9–43.8) |
| BH (45) | 15.1±4.3 (5.2–24.1) | 41.0 ± 5.4 (32.5–58.6) |
| BH %SL (41) | | 22.7 ± 6.0 (14.3–36.6) |
| BH2 (60) | 10.7±3.3 (2.3–19.6) | 29.1 ± 4.8 (20.6–46.2) |
| Minb (62) | 5.6±1.6 (1.8–8.5) | 15.0 ± 2.5 (9.2–23.2) |
| DL (51) | 23.9±6.1 (5.7–37.9) | 63.1 ± 6.5 (44.1–73.1) |
| AL (52) | 7.3±2.0 (2.4–12.7) | 19.1 ± 2.7 (13.6–27.3) |
| VL (23) | 8.2±1.7 (5.1–12.7) | 21.0 ± 4.0 (13.9–31.5) |
| VH (10) | 1.8±0.8 (0.8–3.5) | 4.9 ± 1.2 (3.1–7.5) |
| CL (52) | 9.9±2.8 (3.8–16.0) | 26.7 ± 6.1 (12.5–36.5) |
| Asc (35) | 3.8±1.1 (0.8–5.7) | 10.4 ± 2.4 (4.2–14.7) |
| UJ %HL (22) | 5.3±1.5 (1.6–7.7) | 27.0 ±4.2 (21.2–35.6) |
| LJ %HL (34) | 7.5±2.4 (2.1–12.0) | 39.3 ±5.1 (24.8–48.4) |
| Ped (50) | 12.0±2.8 (3.1–17.3) | 31.5 ±4.1 (16.4–45.3) |
| Vsp (58) | 6.5±2.0 (0.9–10.3) | 17.4 ±2.8 (8.6–24.8) |
| **Meristics** | | |
| Dorsal fin | XI–XIII/6–10 | |
| Anal fin | III/6–10 | |
| Pelvic fin | I/5 | |
| Pectoral fin | 13–15 | |
| Vertebrae | 26–30 (12–15+12–15) | |
| VtPtLDs | 10–15 | |
| Caudal fin | 4–9, 8+8, 4–7 | |

**Abbreviations**: **AL**, length of anal fin base; **Asc**, length of ascending arm of premaxilla; **Asp1–3**, length of anal spines 1–3; **BH**, maximum body depth; **BH2**, body depth at anal fin origin; **BL**, body length; **CL**, length of caudal fin; **DL**, length of dorsal fin base; **Dsp1–13**, length of dorsal spines 1–13; **HH**, head depth; **HL**, head length; **LJ**, length of lower jaw; **Minb**, minimum body depth; **Orbit**, diameter of orbit; **Ped**, length of caudal peduncle; **SL**, standard length; **TL**, total length; **UJ**, length of upper jaw; **VH**, length of pelvic in base; **VL**, length of pelvic fin; **VtPtLDs**, vertebra associated with last dorsal fin spine; **Vsp**, length of pelvic fin spine.

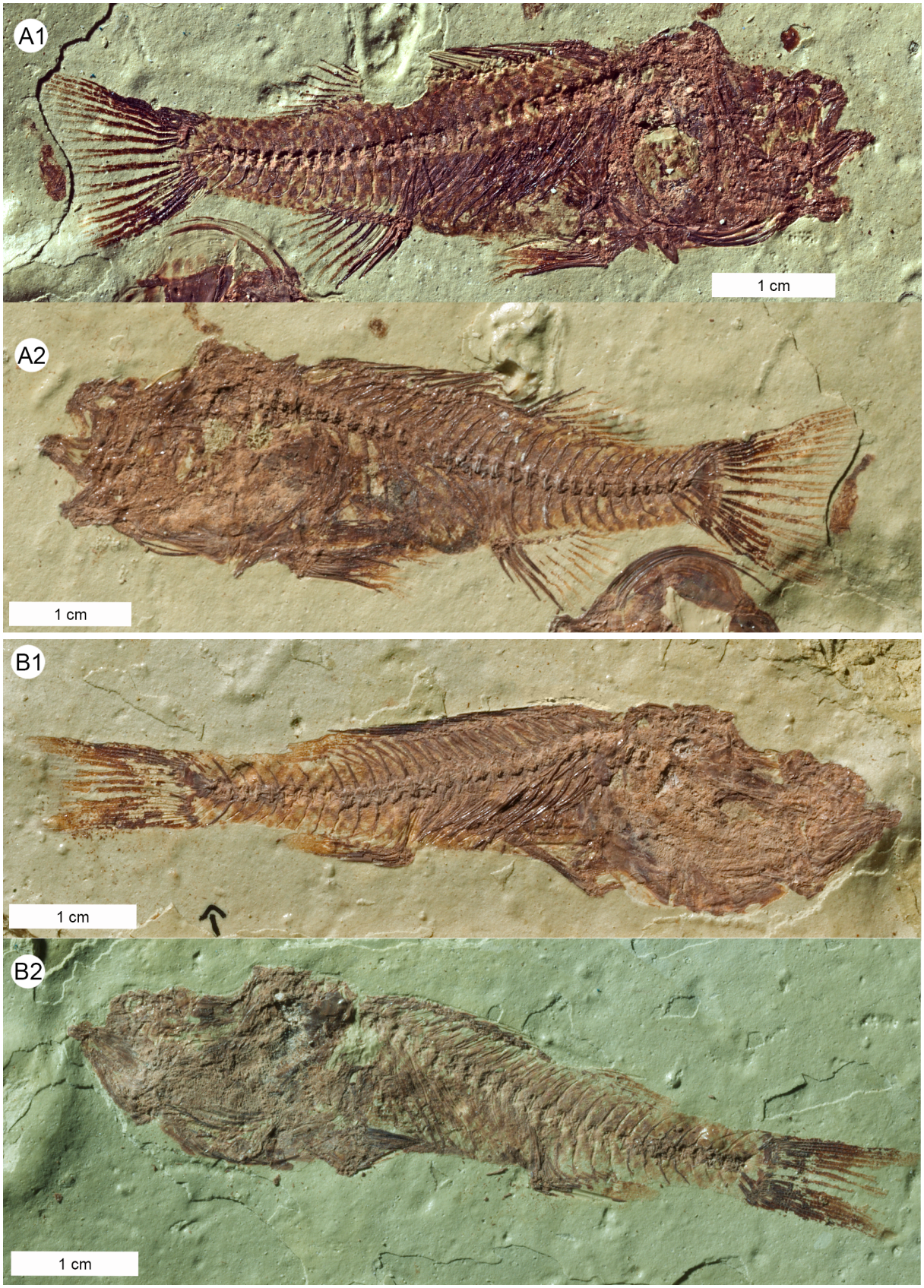


**Figure 1. Type species of †*Baringochromis*, lateral view**. **A1–A2** Holotype of †*B. senutae* sp. nov. (part and counterpart of OCO-5-37/42(1)); **B1–B2** Paratype of †*B. senutae* (part and counterpart of OCO-5-8/23(3)).

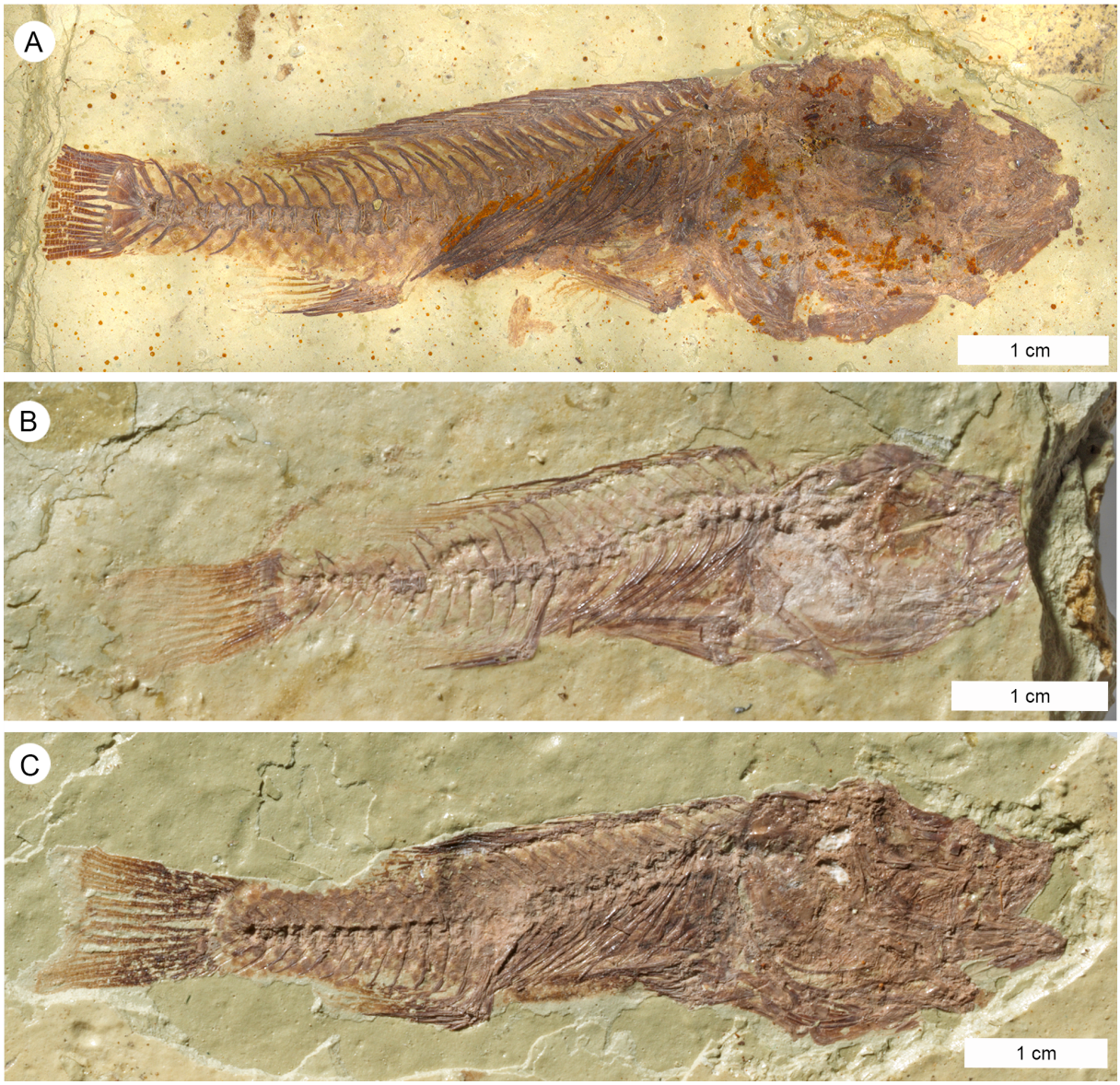


**Figure 2.** †***Baringochromis sonyii*** **sp. nov. A** Holotype, lateral view (2014-WA-19(1)); **B** Paratype, lateral view (2014-WA-9); **C** Paratype, dorsolateral (head) and lateral view (2014- W A-2a).

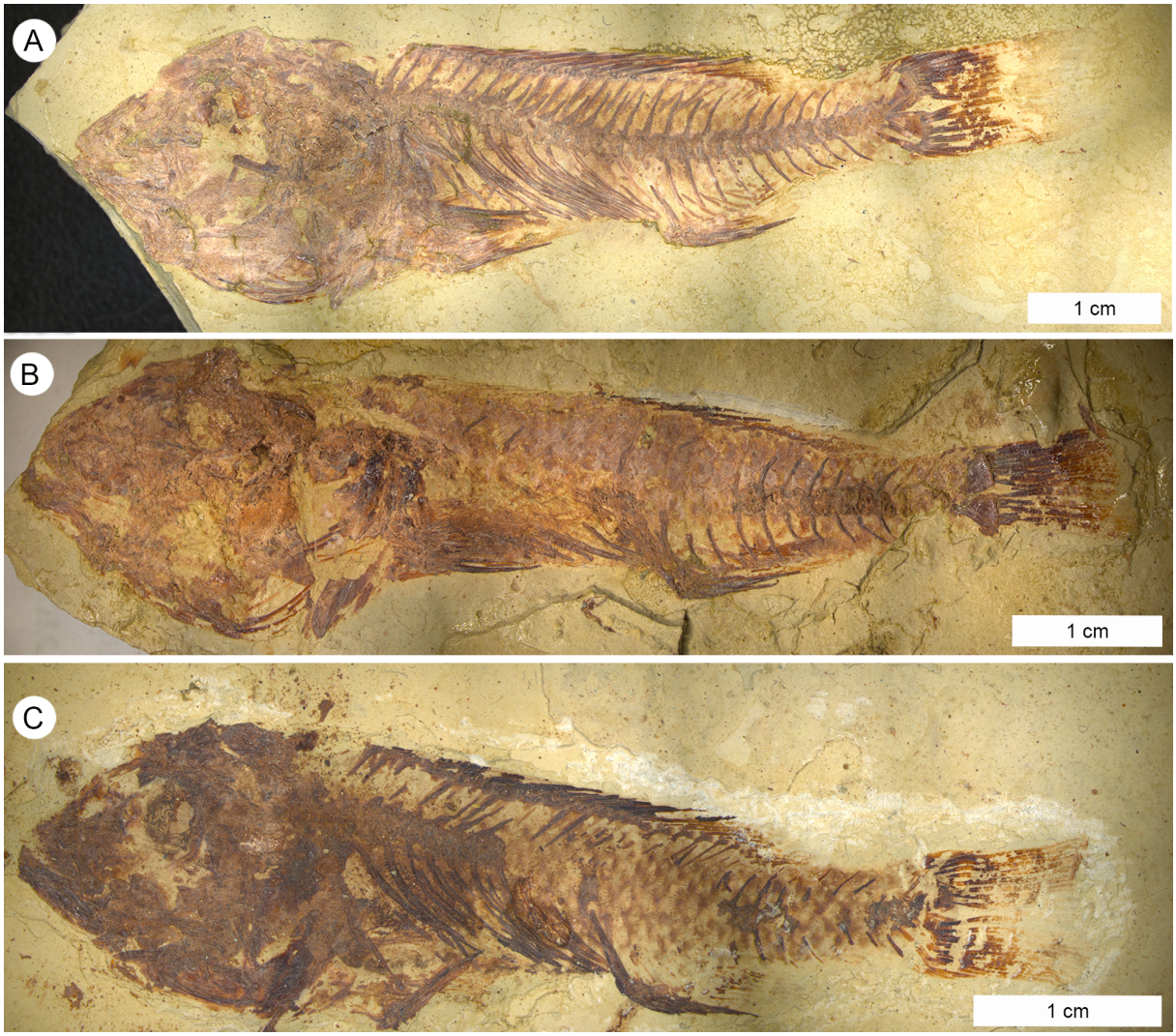


**Figure 3.** †***Baringochromis stellae*** **sp. nov., lateral view A** Holotype (2014-WA-8(1)); **B** Paratype (OCO-5-13(2)); **C** Paratype (OCO-5-31(3)).


**Figure 4. †*Baringochromis davidae* sp. nov., lateral view A** Holotype (2014-WA-16(2)); **B** Paratype (OCO-5b-3b(1)).

**Neurocranium.** The elongated and distally widened nasal bone is preserved lateral to the premaxillary ascending spine (Figs. 5D1, D3–D4, 6A1, A3). The frontals are laterally compressed and elongate (Figs. 5A–B). The parietals are elongate, follow immediately behind the frontals, and are connected to the epiotics by the parietal crest. The supraoccipital crest is low and short, extending to the middle of the orbital diameter (Figs. 5C–D). The orbit is rather small and nearly round, with a vertical diameter of 11.2 ± 2.1% of BL. The parasphenoid bisects the orbit into approximately equal parts. The neurocranial sensory canals (nlc) are visible on the frontals. Whether they meet at the midline or not is unclear, but the supraoccipital crest seems to separate them (Fig. 5A–D).

**Infraorbital series.** Six infraorbitals (io) are visible surrounding the orbit: the lacrimal (io1) and io2–6; the last bone might be the dermosphenotic (Figs. 5D1–D4, 6A1–A3). The lacrimal is quite large and appears to be rectangular in form, but its precise shape is not preserved. The lateral line can be seen to branch into four or five tubules (Figs. 5D1–D4, 6A1–A3). Infraorbitals 2-6 appear as tubular bones with one sensory canal in the middle. Whether the lacrimal overlaps with io2 could not be determined. Io4 and io5 are elongated (Fig. 5D1–D4, 6A1–A3).

**Jaws and teeth.** The ascending arm of the slender premaxilla is shorter than the straight to slightly concave dentigerous arm (10.3 ± 2.5 vs. 13.6 ± 2.5; Figs. 5D3–D4, 6A3, 7B), with an angle of about 90° between them. The maxilla is longer than the dentigerous arm of the premaxilla; its anterior margin is nearly straight, whereas the posterior margin exhibits a pronounced dorsal wing (Figs. 5D1, D3–D4, 6B–C). In dorsal view the premaxillad and palatinad wings of the articular head of the maxilla are visible (Fig.7C) and are widely separated from each other. The dentary is short and robust. Its lower and upper limbs are of approximately equal length and form a posteriorly open triangle, into which the anterior process of the anguloarticular inserts (Figs. 5D3–D4, 6A3, 7A–B). Teeth can be discerned on the first two-thirds of the dentary. The anguloarticular is slightly longer than deep (Figs. 5D3–D4, 6A3, 7A–B), with a pointed, dorsally directed primordial process. The ventralmost part of the anguloarticular (the coulter area in Barel 1976) is longer vertically than horizontally and is closely associated with the small retroarticular. The oral jaws bear prominent, slightly recurved conical or long and slender tricuspid teeth (Fig. 7D); it is not possible to discern how many rows there are. However, one specimen with unicuspid dentition also presents a single tricuspid tooth on its dentary (OCO-5-8/23(6)). We tentatively interpret the unicuspid teeth as the outer row dentition and the tricuspid dentition as the inner row dentition. The teeth on the pharyngeal bones are bicuspid, with a prominent and slightly recurved major cusp and a small minor cusp (Fig. 7F), or simple unicuspid (Fig. 7G). In one specimen the pharyngeal jaws are preserved, but their outline is unclear (Fig. 7E).

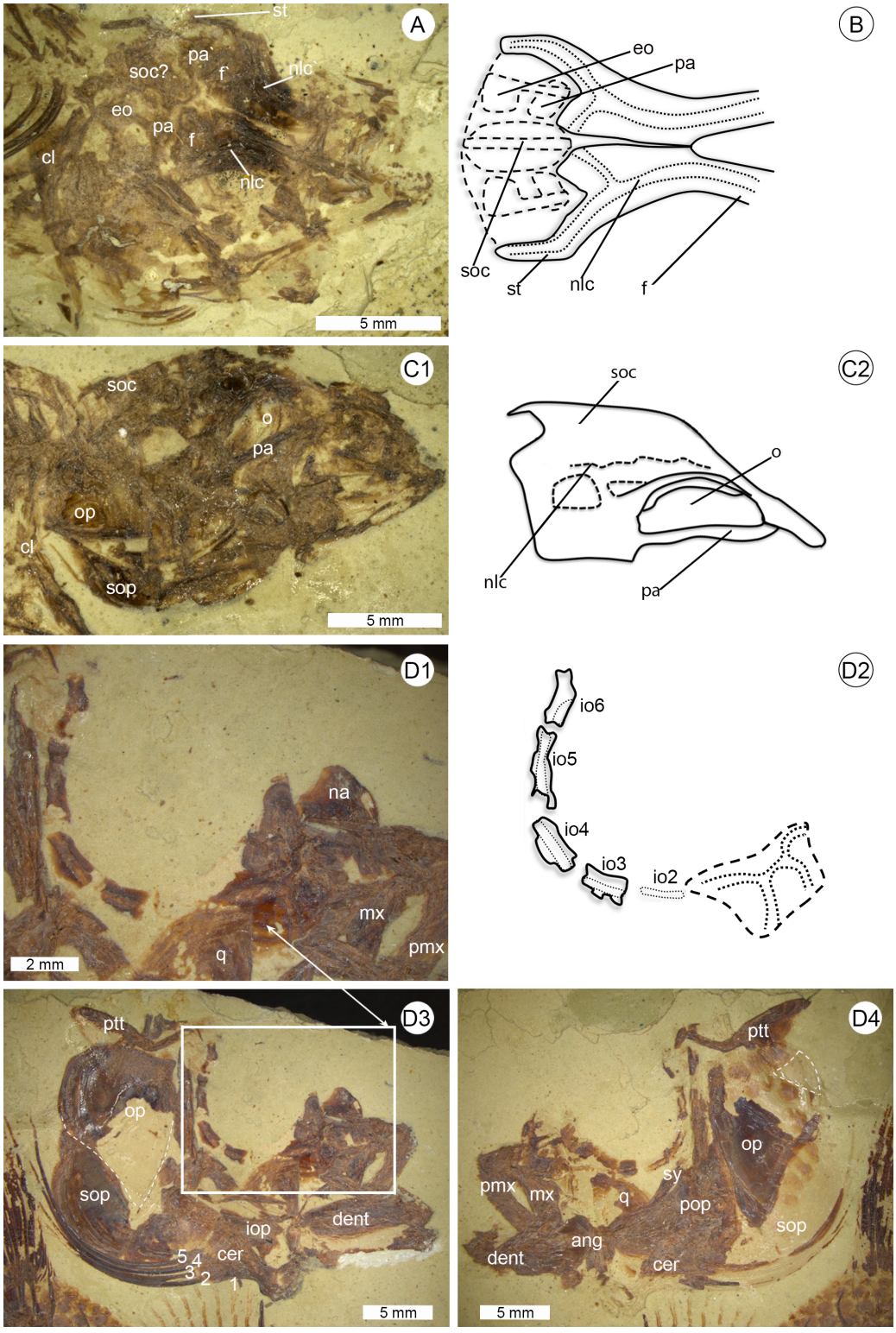


**Figure 5. Neurocranium, infraorbital series and suspensorium of †*Baringochromis* spp. A** head in dorsolateral view showing epiotics, frontals, neurocranial lateral line canals, and parietals (OCO-5-4); **B** general reconstruction of the neurocranium, dorsal view; **C1** head in lateral view showing low supraoccipital process (OCO-5-21(1)); **C2** reconstruction drawing of C; **D1–D2** infraorbital series with lacrimal with four tubules followed by five infraorbitals (OCO-5-37(2)); **D3–D4** head in lateral view showing upper and lower jaws, suspensorium and squamation on operculum and suboperculum (D3 part (OCO-5-37(2)) and D4 counterpart (OCO-5-42(2))). Stippled white line denotes outline of operculum (D3) and opercular blotch (D4). Abbreviations: **ang**, anguloarticular; **cer**, ceratohyal; **cl**, cleithrum; **dent**, dentary; **eo**, epiotic; **f**, frontal; **io1**, lacrimal; **io1–6**, infraorbitals 1–6; **iop**, interoperculum; **mx**, maxilla; **na**, nasal; **nlc**, neurocranial lateral line canal; **o**, orbit; **op**, operculum; **pa**, parasphenoid; **pmx**, premaxilla; **ptt**, posttemporal; **sy**, symplectic; **soc**, supraoccipital process; **sop**, suboperculum; **st**, supratemporal; **1–5**, branchiostegal rays 1–5.

**Suspensorium and hyoid arches.** The quadrate is triangular with a concave posterior margin. Its condyle is anteroventrally directed and articulates with the articular facet of the anguloarticular (Fig. 6A3). The symplectic is a narrow and laminar bone that contacts the quadrate dorsoventrally and extends posteriorly almost as far as the hyomandibula (Fig. 6A3). The L-shaped preoperculum has an elongated and dorsally pointed vertical arm, whereas its horizontal arm is much shorter and thicker; the posteroventral corner is rounded and forms an approximately 90° angle (Fig. 6A3, C1–C2). It presents a branched sensory canal with two terminal and five medial pores. The hyomandibula is found dorsal to the dorsal tip of the preoperculum and extends to the middle of the vertical arm of the preoperculum (Fig. 6A3). The operculum itself is thin and almost triangular in shape, with a pointed anteroventral corner, which is in contact with the subopercular. Its anterior margin is convex and has a pointed anterodorsal process, whereas the posterior margin is S-shaped (Fig. 5D3–D4, 6A3). The subopercular has a curved ventral margin and a prominent pointed ascending process anteriorly, projecting between the operculum and subopercular (Figs. 6A3, B). The interopercular is an elongated and slender element with rounded anterior and posterior ends (Figs. 5D3, 6A3). The ceratohyal bears five branchiostegal rays, of which four are attached to the anterior ceratohyal and the fifth is attached to the posterior ceratohyal (Fig. 5D3). The palatine is not preserved in any of the specimens.

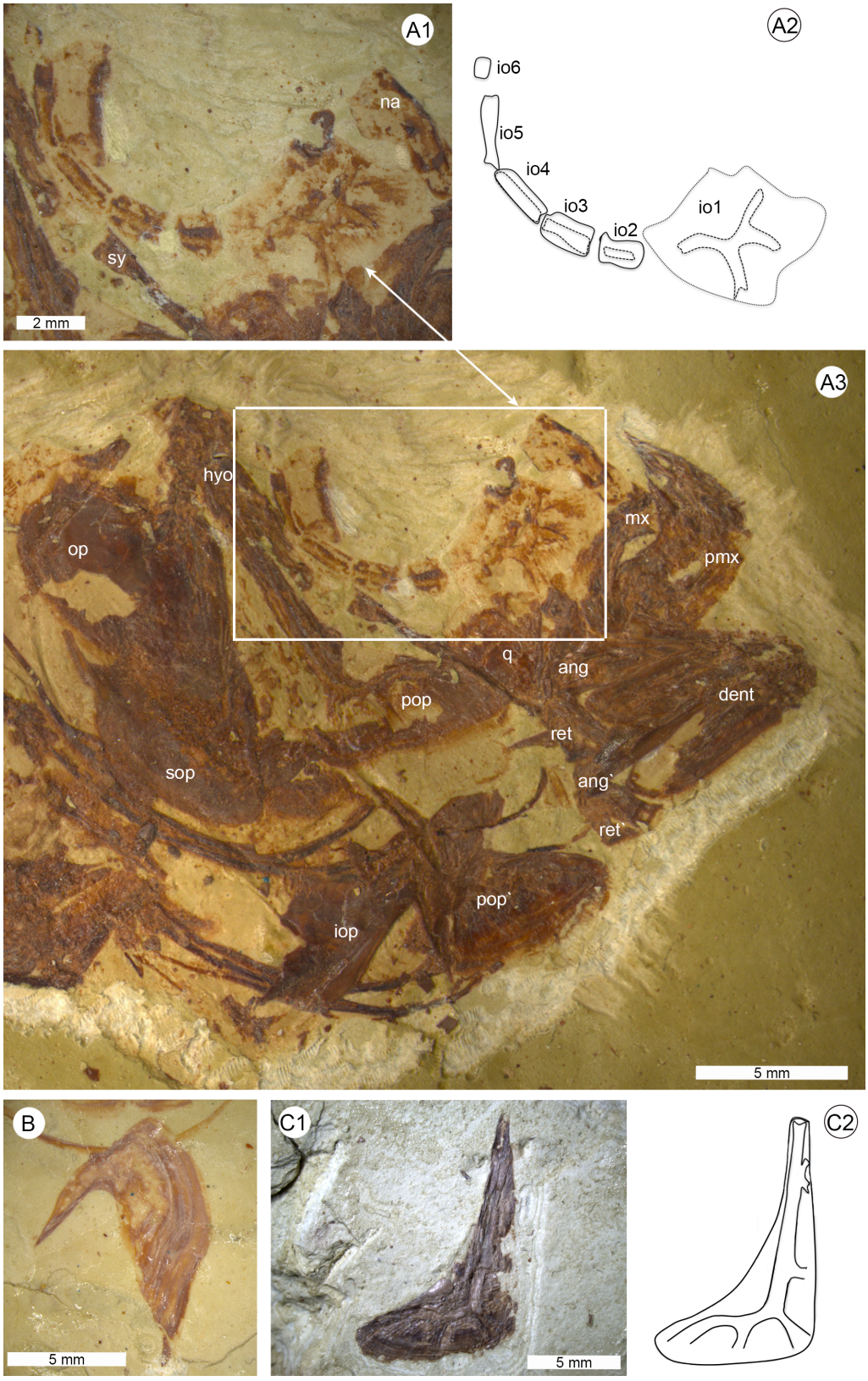


**Figure 6. Characters of the skull and suspensorium of †*Baringochromis* spp. A1** close up of infraorbital series with lacrimal with four tubules followed by five infraorbitals (OCO-5-43); **A2** reconstruction drawing of A1; **A3** overview of head showing operculum, subopercula, preoperculum, infraorbital series, dentary, angular, premaxilla (OCO-5-43); **B** isolated subopercula (OCO-5b-10(1)); **C1** isolated preoperculum (OCO-5-23R(9)); **C2** reconstruction drawing of C1. **Abbreviations**: **ang**, anguloarticular; **dent**, dentary; **hyo**, hyomandibula; **io1**, lacrimal; **io2–6**, infraorbitals 2–6; **iop**, interoperculum; **mx**, maxilla; **na**, nasal; **op**, operculum; **pmx**, premaxilla; **ret**, retroarticular; **sy**, symplectic **sop**, subopercula.

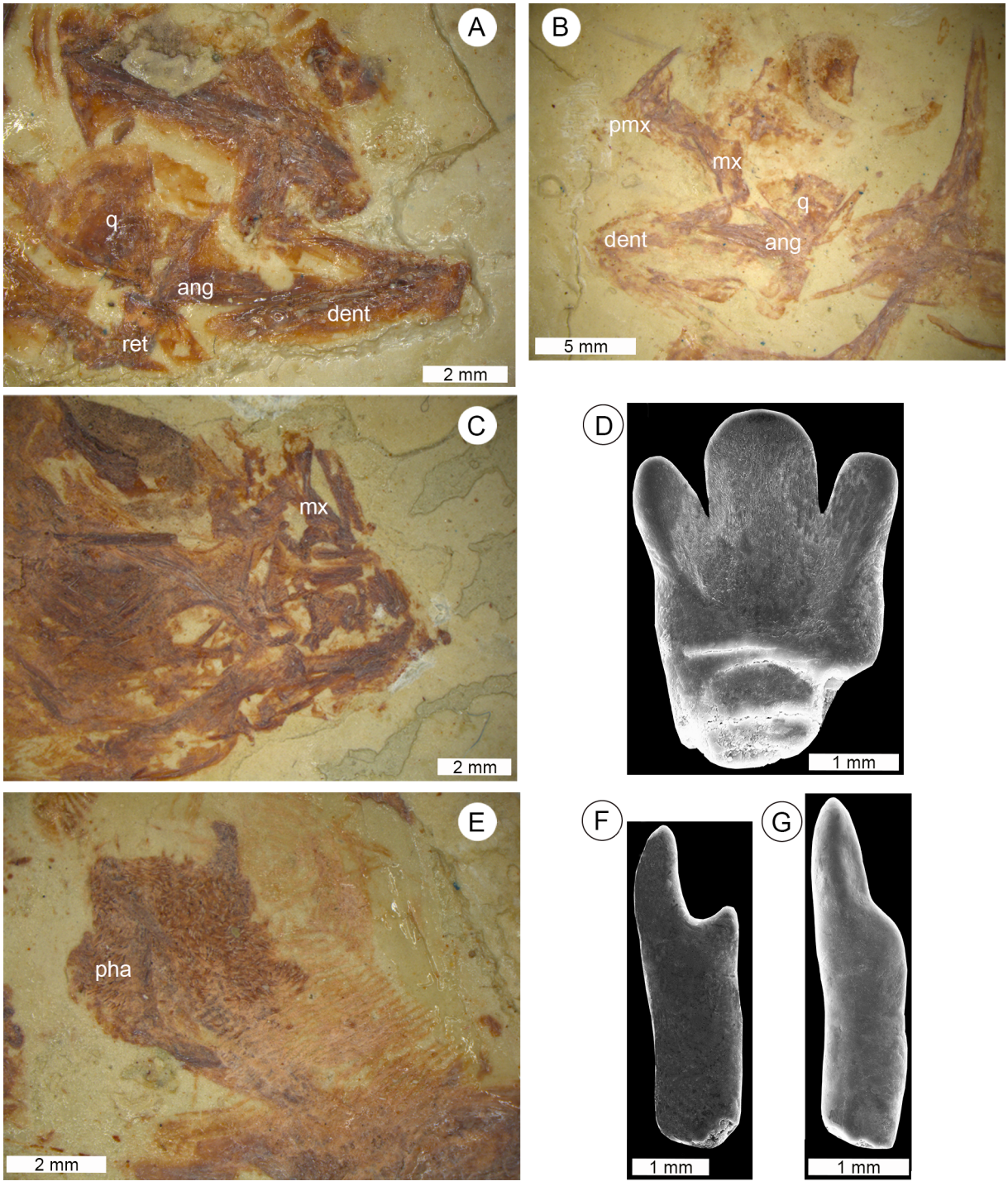


**Figure 7. Jaws and teeth of †*Baringochromis* spp. A** close up of lower jaw with anguloarticular, retroarticular and quadrate (OCO-5-1(1)); **B** close up of upper and lower jaws (OCO-5b-10(1)); **C** head in dorsal view showing maxillary wings (OCO-5-5(1)); **D** tricuspid tooth (OCO-5-23R(2)); **E** pharyngeal jaw with pharyngeal teeth in situ (OCO-5-8(7)); **F** bicuspid pharyngeal tooth (OCO-5-19); **G** unicuspid pharyngeal tooth (OCO-5-42(1)). **Abbreviations**: **ang**, anguloarticular; **dent**, dentary; **mx**, maxilla; **pha**, pharyngeal jaw; **pmx**, premaxilla; **q**, quadrate; **ret**, retroarticular.

**Vertebral column.** The vertebral column is gently curved and contains a total of 26-30 vertebrae; 12–15 of them are abdominal and 12–15 caudal (see Table 1). All vertebral centra bear a longitudinal lateral ridge (Fig. 1). The first two and the last two vertebrae are short. The first, and in some cases the second, neural spine project in front of the first dorsal pterygiophore. The neural spines are short at the beginning of the vertebral column, gradually increase in length towards the end of the spinous part of the dorsal fin and shorten again along the caudal peduncle (Figs. 1–4). There are 10–13 pairs of robust ribs, which reach the margin of the abdominal cavity and are connected to the centra by strong parapophyses. The first pair of ribs originates on the third vertebra. A single predorsal bone is present between the supraoccipital and the first pterygiophore. Epineurals are not recognizable (Figs. 1–4).

**Median fins and support.** The caudal skeleton is similar to that of other members of the Cichlidae. It includes five hypural plates, a parhypural, two epurals, one uroneural, and one or two preural vertebrae (Fig. 8). The condition of the hypural plates is recognizable in 66 specimens. Hypural plates 1 and 2 and hypural plates 3 and 4 can either be fused or be separated by a suture. In the latter case, hypural plate 1 is always larger than hypural 2 and hypural 4 is always larger than hypural 3. Hypural 5 is slender and always separate from hypural 4. It extends into the space between the urostyle and the uroneural. The diastema is small, ranging from 0.2 to 1.2 mm in depth. The parhypural is broad and its distal section lies close to hypural 1; it can either be isolated from, or make contact with the urostyle, and displays a short, posteriorly directed hypurapophysis on its proximal part (Fig. 8).

The uroneural is long (half the length of the neural spine of PU3) and extends between the second epural and hypural 5. Two elongate epurals of equal length and width are aligned in parallel between the uroneural and the neural spine of PU3. PU2 lacks a neural spine, while the neural arch is present. The urostyle (preural centrum 1) is approximately triangular in shape, and extends posteriorly so that it separates hypurals 4 and 5.

The caudal fin has a slightly rounded to truncated posterior margin and is made up of 16 (8 dorsal + 8 ventral) principal rays and 4–9 dorsal and 4–7 ventral procurrent rays. The principal fin rays are aligned without interruption and supported by the parhypural, the epurals and the five hypurals.

The dorsal fin consists of 11–13 spines and 6–10 rays (see Table 1), with the spiny and soft dorsal fin portions being continuous. The spiny dorsal fin base is up to three times longer than the rayed one (Figs. 1–4). The anteriormost spines increase in length from the first to the last. They are followed by segmented rays, which are longer than the spines, sometimes even

reaching the caudal fin. Each spine and ray is supported by a thin and elongate pterygiophore, with exception of the last ray, which can be autogenous. Every pterygiophore is associated with its individual interneural space. The first pterygiophore inserts into the interneural space between vertebrae 1 and 2 or 2 and 3, while the last pterygiophore associated with a dorsal spine inserts behind the neural spine of vertebra 10, 11, 12, 13, 14 or 15 (see character VtPtLDs in Table 1). The pterygiophores associated with the rays gradually shorten towards the caudal fin (Figs. 1–4).

The anal fin originates approximately far behind the dorsal fin origin (Figs. 1–4). It consists of three spines and 6–10 segmented rays (see Table 1), which are longer than the spines and sometimes reach the caudal fin. The two anteriormost fin spines are supported by one pterygiophore, while the third spine and the branched rays are each associated with a single pterygiophore, though the last ray can be autogenous. The first pterygiophore is associated with the haemal spine of the first caudal vertebra or the rib of the last abdominal vertebra. The anal fin spines increase in length posteriorly, the third being the longest (2.1–2.6 times the length of the first; see Table 1). The first three branched rays are the longest ones; they gradually diminish in size, as do the pterygiophores (Figs. 1–4).

**Paired fins and support.** The cleithrum is elongate and curved, with a lamellar posterior projection and a pointed ventral end. A small and pointed process is present at the posteroventral extension (Figs. 9A1–A2). The dorsal process is not recognizable; it is most probably overlain by the supracleithrum. The posttemporal is bifurcated, with the upper limb thinner than, but approximately as long as the lower (Fig. 9A1–A2). An elongated and slender supracleithrum is visible at the dorsal end of the cleithrum, covering the upper end of the latter. The scapula is rectangular with a central scapular foramen and supports the upper two proximal radials of the pectoral fin. The coracoid is cone-shaped, tapers rostrally and supports the lower two of the four rectangular proximal radials of the pectoral fin. The uppermost of the proximal radials is the smallest and the lowermost the largest (Fig. 9B). The pectoral fin has 13–15 rays and the ventralmost rays diminish continuously in size, giving this fin a pointed shape (Fig. 9A1).

The pelvic plate is triangular and anteriorly tapered. The pelvic fin has one spine and five rays, none of which reach the anal fin (Fig. 9C).

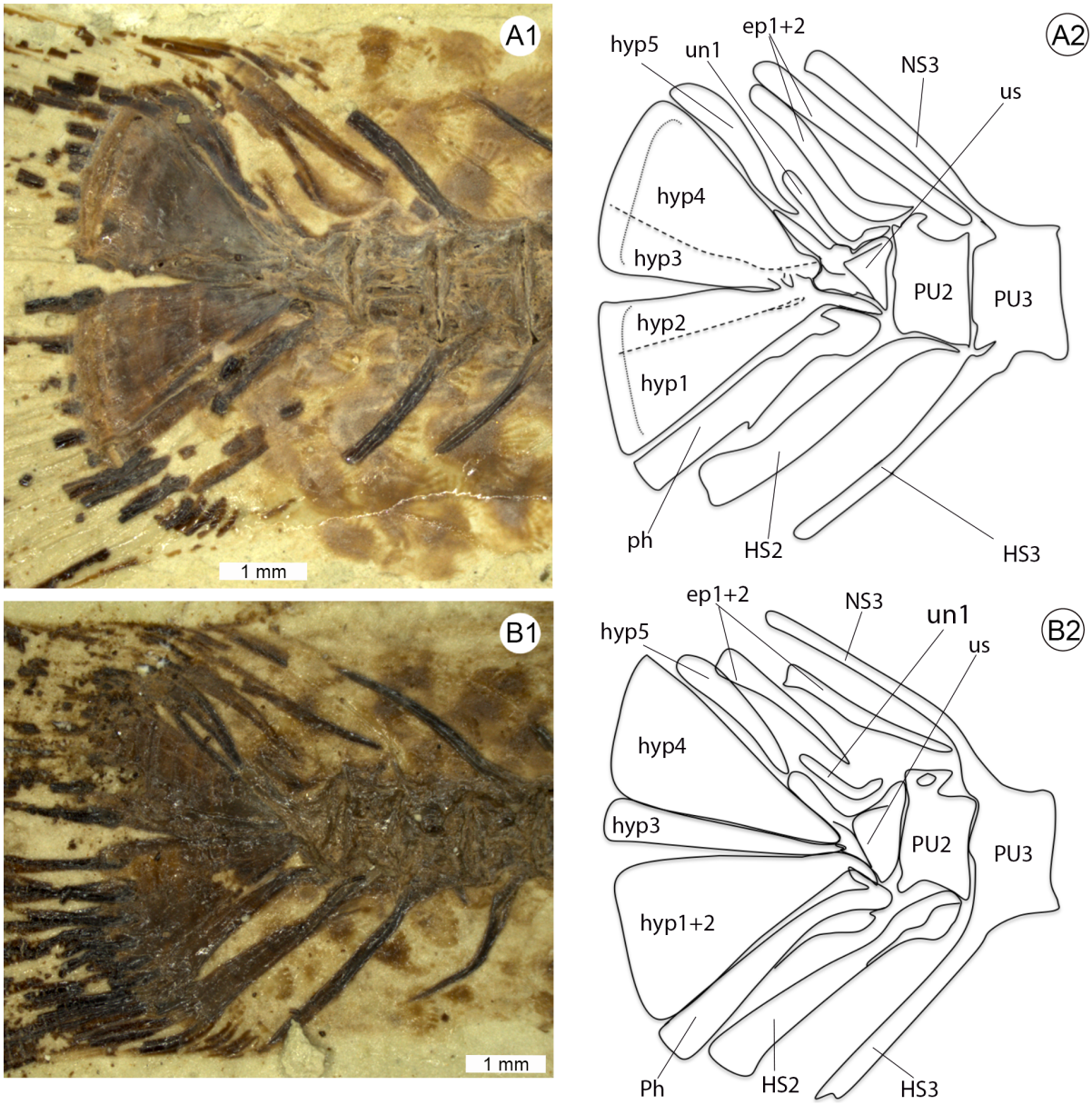


**Figure 8. Caudal fin of †*Baringochromis* spp. A1–A2** caudal skeleton showing hypural plates 1 and 2, and 3 and 4 separated by a suture (OCO-5-5(2)); **B1–B2** caudal skeleton showing hypural plates 1 and 2 fused and 3 and 4 well separated (OCO-5-8(6)). **Abbreviations**: **ep1+2**, epural 1 and 2; **HS2–3**, haemal spines of preural vertebra 2 and 3; **hyp1–5**, hypural plates 1– 5; **NS3**, neural spine of preural vertebra 3; **PU2–3**, preural vertebrae 2–3; **us**, urostyle; **un1**, uroneural 1.

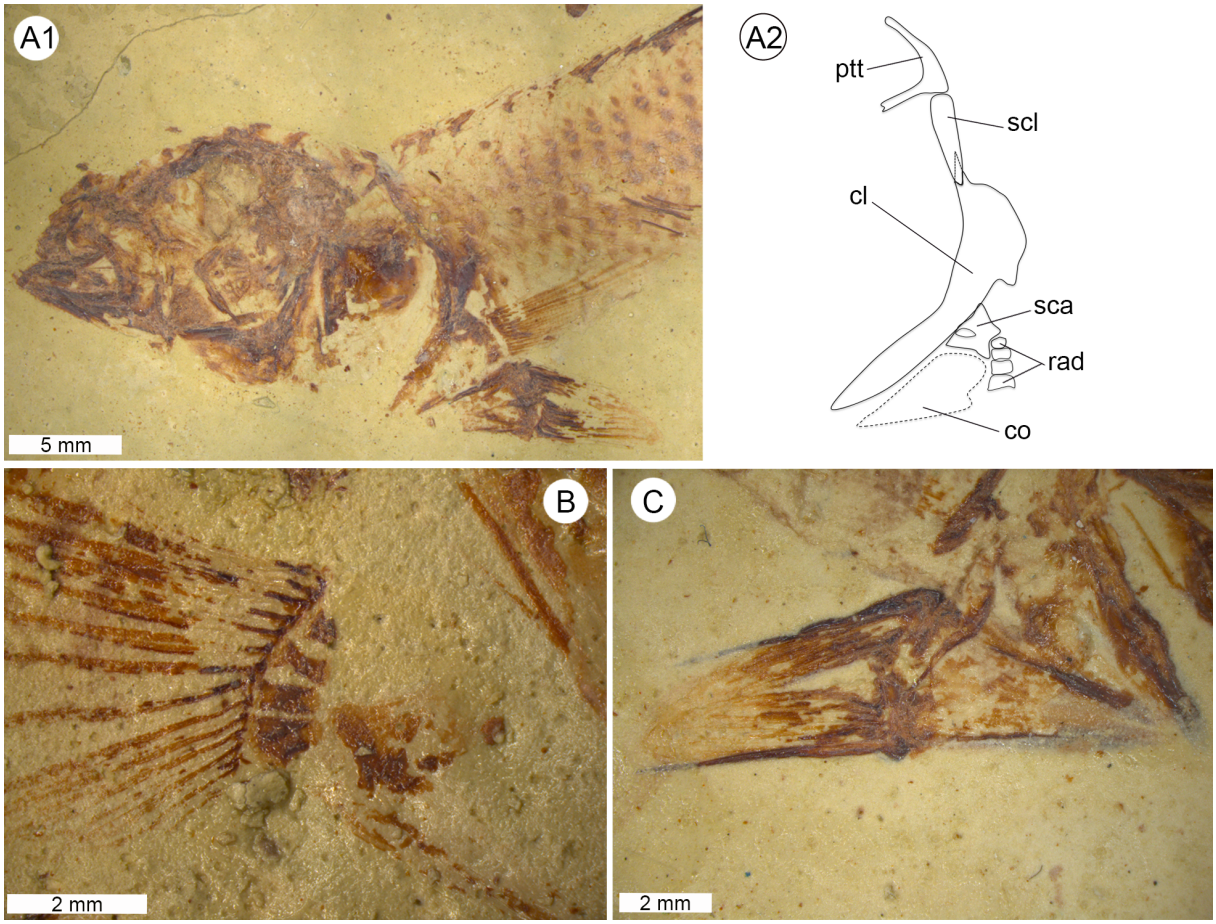


**Figure 9. Pectoral and pelvic girdle of †*Baringochromis* spp. A1** pectoral girdle (OCO-5- 21(19); **A2** reconstruction drawing of A1; **B** pectoral fin with 14 fin rays and four proximal radials (OCO-5-38R(5)); **C** pelvic girdle and fins (OCO-5-15(1)). **Abbreviations**: **cl**, cleithrum; **co**, coracoid; **ptt**, posttemporal; **rad**, proximal radials; **sca**, scapula; **scl**, supracleithrum.

**Squamation.** Large, ovate (1.32–1.64 mm height and 1.47–2.04 mm width) cycloid scales cover the body and head. The preopercle and the interopercle appear to be scaleless. The opercle bears large cycloid scales (Fig. 5D4). The subopercle is covered by a single row of large cycloid scales, but is scaleless ventrally (Fig. 5D4). Small scales are present on and in between the fin rays in the lower fourth of the caudal fin. No scales are recognizable on the occiput or prepelvic region.

As is typical for cichlids, the lateral line is divided into two parts (Fig. 10A). The origin of the anterior trunk canal segment is not recognizable, but it seems to consist of approximately 15 scales (not all of which are pored) with the posterior end located approximately above the 20th vertebra. There is a gap of two to three scale rows between the anterior and posterior trunk canal segments, while 1.5 to two scale rows lie between the anterior trunk canal segment and the dorsal fin. The posterior trunk canal segment (consisting of 10 to 11 pored scales) continues approximately opposite to or slightly behind the end of the anterior trunk canal segment and runs either above, below (Fig. 10B), or on the vertebral column (Fig. 10A). The flank scales show up to 14 radii (Figs. 10B–C).

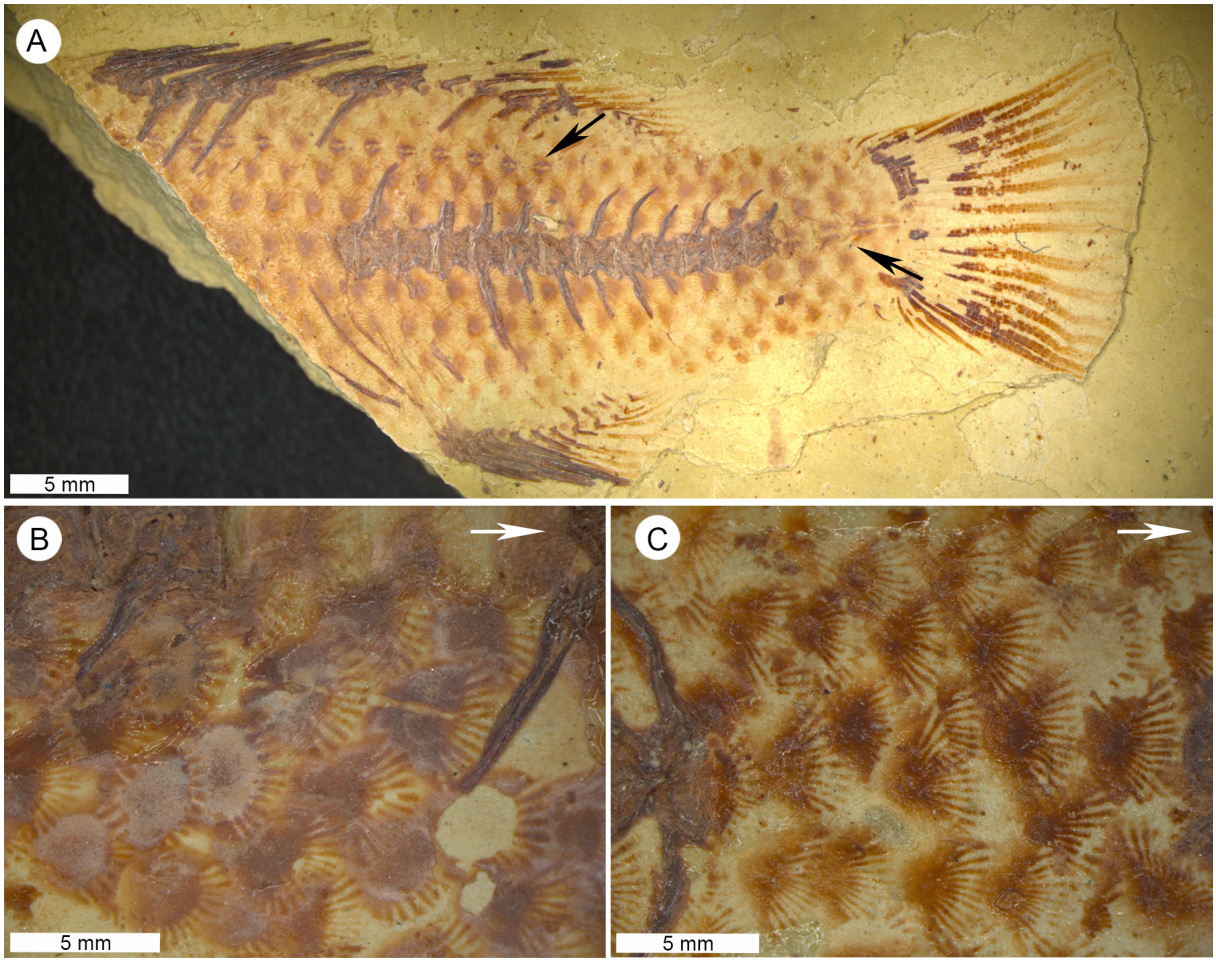


**Figure 10. Squamation of †*Baringochromis* spp. A** divided lateral line (arrows) with high-set anterior trunk canal segment (>13 tubular scales) and posterior trunk canal segment on the vertebral column (> 4 tubular scales; OCO-5-9); **B** normal and lateral line-scales of the posterior trunk segment below the vertebral column on the peduncle showing radii and tubular sensory opening; arrow points anteriorly (OCO-5-5(2)); **C** cycloid scales on transition zone between abdominal and caudal part; arrow points anteriorly (OCO-5-8(7)).

**Otoliths.** A pair of saccular otoliths was found in an isolated head of †*Baringochromis* sp. (OCO-5-23R(2)). The otoliths are of elliptical shape and have smooth to slightly crenate margins (Figs. 11A1–A4). The inner face is planar to very slightly convex and the outer face is almost planar. A prominent and pointed rostrum and a much shorter antirostrum is present, with a deep excisura between them. The ventral margin is slightly curving, the posterior margin is round to blunt, and the dorsal margin has a median tip with a slight indentation behind it. The sulcus is in median position; it first runs straight before curving downwards in its posterior section. The ostium is wider than the cauda; the ostium is deep, the cauda less so, but still deep. The crista inferior is weak, whereas the crista superior is high and sharp along the ostium and the cauda, with the exception of the posteriormost segment of the cauda. The crista inferior is very thin. The ventral line is relatively high set.

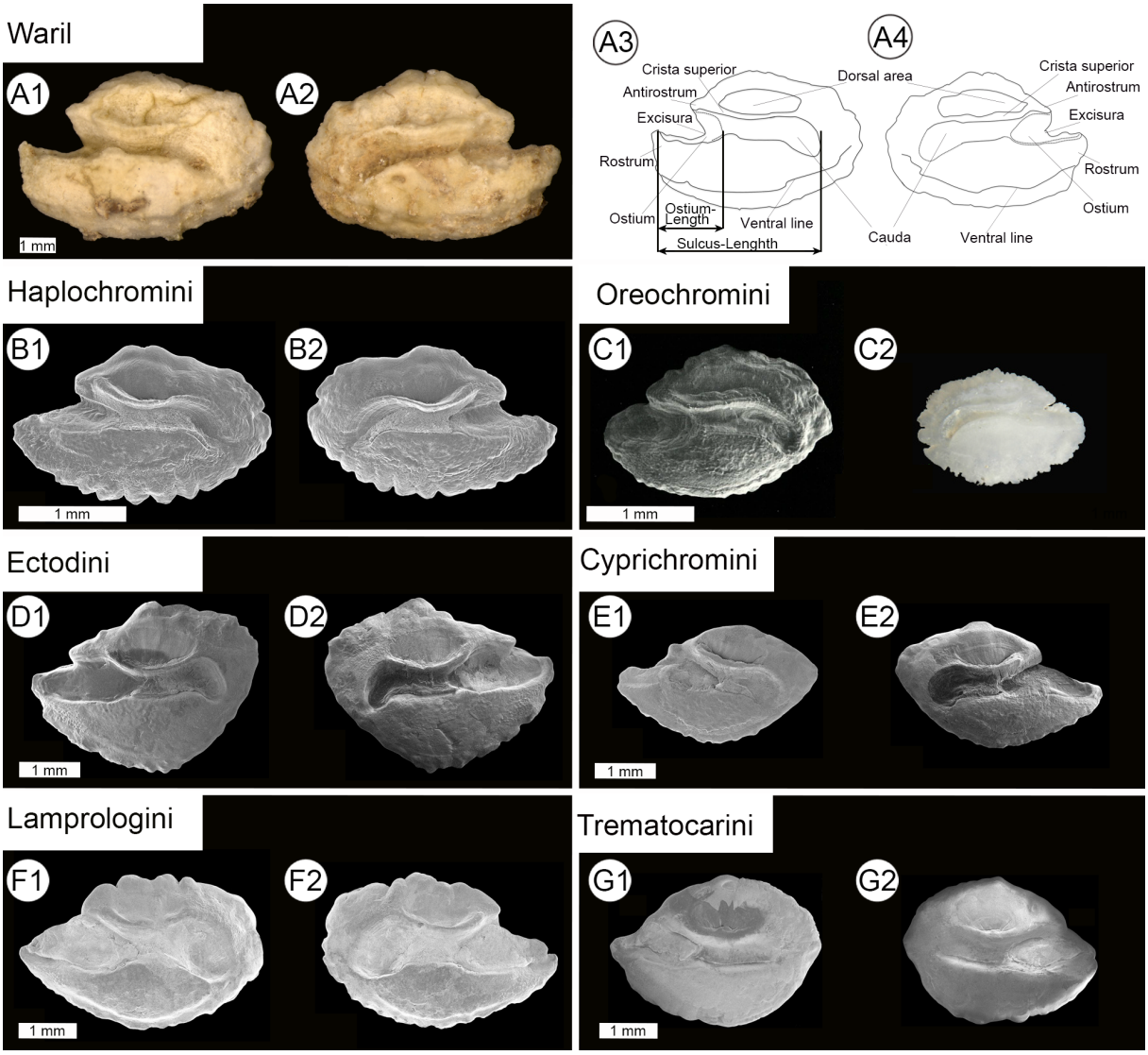


**Figure 11. Otoliths of †*Baringochromis* and recent cichlids. A1–A2** otoliths found in situ in †*Baringochromis* sp. (OCO-5-23R(2)); **A3–A4** reconstruction drawing with terminology and measurements; **B1–B2** right and left sagitta of *Tropheus duboisi* (NHM 2011/0227/0382); **C1** right sagitta of *Oreochromis latilabris* (modified from Tichy and Seegers (1999)); **C2** left sagitta of *Oreochromis aureus* (modified from Artzi (2009)); **D1–D2** right and left sagitta of *Ophthalmotilapia ventralis* (ZSM 043241_(P-AA-1001)); **E1–E2** right and left sagitta of *Cyprichromis leptosoma* (ZSM 043238_(P-AA-0996), left & ZSM 043238_(P-AA- 0995), right); **F1–F2** right and left sagitta of *Neolamprologus tetracanthus* (NHM 2011/0227/0356); **G1–G2** right and left sagitta of *Trematocara marginatum* (ZSM 039570).

### 4.3.2. Description of species of †*Baringochromis*

**Preliminary remark:** Quantitative comparison between species was performed on the basis of non-parametric tests (SPSS Inc. 23, $p<0.01$), as not all characters were normally distributed, probably because of the small sample sizes.

†*Baringochromis senutae* sp. nov.

(Fig. 1)

**Holotype.** OCO-5-37(1) and OCO-5-42(1) (part and counterpart).

**Paratypes.** Six skeletons (OCO-5-8/23(3), -5-29(1), -5-29R(1), 5-31(1), -5b-6, 2014-WA-7(2)). [(n) indicates an individual specimen when more than one specimen is preserved on the same slab; '/' indicates presence of part and counterpart]

**Locality, horizon and age.** Outcrop Waril (0°40′56.21′′N 35°43′7.43′′E) in Central Kenya; Ngorora Formation, member E; late Miocene (9-10 Ma) (see Rasmussen et al., 2015).

**Diagnosis.** Distinguished from other species of the genus †*Baringochromis* by the frontals being sharply bent towards the orbit, creating a pronounced concavity from the tip of the premaxillary ascending process to the posterior margin of the orbit. The lacrimal has the lateral line branched into four to five tubular tubules.

**Etymology.** Species named in honour of Prof. Dr. Brigitte Senut, Paris, for her dedicated research in the field of human evolution and palaeoanthropology on the African continent.

**Comparative description**— †*Baringochromis senutae* has a significantly longer peduncle than †*Baringochromis stellae* ($p<0.001$). All other characters are as described for the genus. Measurements and meristic counts are given in Table 2.

**Table 2. Morphometric measurements and meristic counts of the holotype and designated paratypes of *Baringochromis senutae* sp. nov.**

| Character | Mean±SD (Range) mm | Mean±SD (Range) %BL |
|---|---|---|
| TL (5) | 67.0 ±10.1 (57.4–84.1) | 177.0±16.3 (158.5–194.5) |
| SL (7) | 58.7 ±8.7 (50.8–73.5) | 150.0±10.4 (137.2–161.9) |
| BL (7) | 39.3 ±6.8 (33.2–52.2 | – |
| HL (7) | 19.3 ±3.5 (14.6–24.2) | 50.0±10.3 (37.6–61.9) |
| HH (6) | 18.2±2.4 (15.7–21.3) | 45.9±5.5 (40.8–55.4) |
| Orbit %HL (2) | 20.9±1.6 (19.8–22.1) | 20.9±1.6 (19.8–22.1) |
| BH (6) | 16.5±2.7 (13.9–19.9) | 41.4±4.8 (38.0–49.7) |
| BH2 (7) | 11.5±2.2 (9.5–15.4) | 29.3±3.6 (25.2–34.8) |
| Minb (7) | 6.3±0.9 (5.5–7.7) | 16.2±1.8 (14.0–19.0) |
| DL (7) | 24.5±3.2 (20.2–28.6) | 63.0±7.1 (54.4–73.1) |
| AL (7) | 7.6±1.1 (6.4–9.5) | 19.5±1.8 (16.2–21.8) |
| VL (5) | 8.7±1.5 (6.8–10.5) | 23.2±4.8 (18.9–31.5) |
| VH | | |
| CL (5) | 11.4±2.1 (8.0–13.3) | 30.2±6.4 (22.0–36.5) |
| Asc (3) | 4.3±0.2 (4.1–4.4) | 12.3±0.9 (11.7–13.3) |
| UJ %HL (3) | 5.3±1.2 (4.6–6.7) | 29.8±2.0 (27.6–31.4) |
| LJ %HL (5) | 7.7±1.7 (5.6–9.7) | 41.0±3.7 (37.6–46.4) |
| Ped (7) | 13.1±2.2 (11.3–17.3) | 33.4±1.7 (31.7–36.5) |
| Vsp (6) | 7.1±1.2 (6.0–9.2) | 17.6±7.2 (16.2–19.8) |
| **Meristics** | | |
| Dorsal fin | XI–XIII/8–10 | |
| Anal fin | III/8 | |
| Pelvic fin | I/? | |
| Pectoral fin | 14 | |
| Vertebrae | 27–29 (13–15+12–15) | |
| VtPtLDs | 12–14 | |
| Caudal fin | 5–7,8+8,4–7 | |

**Abbreviations**: **AL**, length of anal fin base; **Asc**, length of ascending arm of premaxilla; **Asp1–3**, length of anal spines 1–3; **BH**, maximum body depth; **BH2**, body depth at anal fin origin; **BL**, body length; **CL**, length of caudal fin; **DL**, length of dorsal fin base; **Dsp1–13**, length of dorsal spines 1–13; **HH**, head depth; **HL**, head length; **LJ**, length of lower jaw; **Minb**, minimum body depth; **Orbit**, diameter of orbit; **Ped**, length of caudal peduncle; **SL**, standard length; **TL**, total length; **UJ**, length of upper jaw; **VH**, length of pelvic in base; **VL**, length of pelvic fin; **VtPtLDs**, vertebra associated with last dorsal fin spine; **Vsp**, length of pelvic fin spine.

†*Baringochromis sonyii* sp. nov.

(Fig. 2)

**Holotype**. 2014-WA-19(1)

**Paratypes.** 13 skeletons (OCO-5-16(4), -5-19, -5-20(7), -5-24(1), -5-30, -5-38(6), -5b-5(1), -4-1, 2014-WA-2a/b(1), -9, -21a/b(1), -22, -25(1)).

**Locality, horizon and age.** Outcrop Waril (0°40´56.21´´N 35°43´7.43´´E) in Central Kenya; Ngorora Formation, member E; late Miocene (9-10 Ma) (see Rasmussen et al., 2015).

**Diagnosis**. Distinguished from other species of the genus †*Baringochromis* by straight frontals, resulting in a sloped anterodorsal profile of the head of approximately 40°, and a slender body.

**Etymology.** Species named in honour of Stefan Sónyi, Munich, Germany, for his commitment and valuable help during fieldwork in Central Kenya and in acknowledgement of his excellent preparation of the fossil fish specimens.

**Comparative description.** †*Baringochromis sonyii* has a significantly longer caudal peduncle than †*Baringochromis stellae* ($p<0.009$). All other characters are as described for the genus. The lacrimal bears four tubules. Measurements and meristic counts are given in Table 3.

**Table 3. Morphometric measurements and meristic counts of the holotype and designated paratypes of *Baringochromis sonyii* sp. nov. (), number of specimens.**

| Character | Mean±SD (Range) mm | Mean±SD (range) %BL |
|---|---|---|
| TL (13) | 69.7±9.3 (58.3–88.5) | 176.9±6.6 (165.1–185.7) |
| SL (14) | 60.1±7.3 (49.6–73.8) | 152.0±3.9 (144.7–156.9) |
| BL (14) | 39.6±4.8 (33.1–49.8) | 100.0± |
| HL (14) | 20.3±3.0 (14.9–24.7) | 51.4±4.6 (40.5–56.6) |
| HH (10) | 17.3±2.0 (13.7–19.7) | 43.3±3.5 (36.6–49.2) |
| Orbit %HL (3) | 21.5±3.0 (18.5–24.4) | 21.5±3.0 (18.5–24.4) |
| BH (13) | 15.1±2.3 (11.1–19.4) | 38.3±4.1 (32.5–43.1) |
| BH2 (14) | 10.2±1.3 (7.6–12.8) | 26.0±3.0 (20.6–29.8) |
| Minb (14) | 5.5±0.8 (3.7–6.7) | 13.8±1.6 (10.9–16.0) |
| DL (12) | 24.5±4.0 (16.1–31.1) | 61.8±8.5 (44.1–70.1) |
| AL (14) | 7.6±1.2 (5.7–9.5) | 19.4±2.5 (13.6–22.9) |
| VL (5) | 6.9±0.2 (6.6–7.1) | 17.6±2.8 (13.9–21.6) |
| VH ( | – | – |
| CL (13) | 11.0±2.4 (7.7–15.7) | 27.9±4.0 (19.5–32.6) |
| Asc (8) | 3.9±0.9 (2.7–5.2) | 9.7±1.8 (6.9–12.2) |
| UJ %HL (5) | 5.5±0.6 (4.6–6.3 | 25.3±2.6 (22.6-29.4) |
| LJ %HL (9) | 8.2±1.9 (5.3–10.9) | 38.7±4.8 (30.9–44.0) |
| Ped (14) | 13.1±1.8 (10.4–15.9) | 33.3±4.3 (27.2–45.3) |
| Vsp (12) | 7.0±1.4 (4.9–9.6) | 17.5±1.8 (14.1–20.4) |
| Meristics | | |
| Dorsal fin | XII–XIII/8–10 | |
| Anal fin | III/7–9 | |
| Pelvic fin | I/5 | |
| Pectoral fin | 13 | |
| Vertebrae | 27–28 (13–15+13–15) | |
| VtPtLDs | 12–13 | |
| Caudal fin | 4–7,8+8,4–7 | |

**Abbreviations**: **AL**, length of anal fin base; **Asc**, length of ascending arm of premaxilla; **Asp1–3**, length of anal spines 1–3; **BH**, maximum body depth; **BH2**, body depth at anal fin origin; **BL**, body length; **CL**, length of caudal fin; **DL**, length of dorsal fin base; **Dsp1–13**, length of dorsal spines 1–13; **HH**, head depth; **HL**, head length; **LJ**, length of lower jaw; **Minb**, minimum body depth; **Orbit**, diameter of orbit; **Ped**, length of caudal peduncle; **SL**, standard length; **TL**, total length; **UJ**, length of upper jaw; **VH**, length of pelvic in base; **VL**, length of pelvic fin; **VtPtLDs**, vertebra associated with last dorsal fin spine; **Vsp**, length of pelvic fin spine.

†*Baringochromis stellae* sp. nov.

(Fig. 3)

**Holotype.** 2014-WA-8(2).

**Paratypes.** Eight skeletons (OCO-5-10/12(1), -5-13(2), -5-16(2), -5-31(3), -5-40(6), -5b-8, 2014-WA-10(1), -20).

**Locality, horizon and age.** Outcrop Waril (0°40′56.21′′N 35°43′7.43′′E) in Central Kenya; Ngorora Formation, member E; late Miocene (9-10 Ma) (see Rasmussen et al., 2015).

**Diagnosis.** Distinguished from other species of the genus †*Baringochromis* by a pronounced and approximately round head (as long as it is deep), with the frontals sloping upwards by more than 50°. The lacrimal shows the lateral line branching into four tubules.

**Etymology.** Species named after Mrs Stella Tallam, Kenya, who significantly contributed to our fieldwork and excavations of fish fossils.

**Comparative description.** †*Baringochromis stellae* has a significantly shorter caudal peduncle compared to †*Baringochromis senutae* ($p<0.001$). The lacrimal bears four tubules. All other characters are as described for the genus. Measurements and meristic counts are given in Table 4.

**Table 4. Morphometric measurements and meristic counts of the holotype and designated paratypes of *Baringochromis stellae* sp. nov.**

| ID | Mean±SD (Range) mm | Mean±SD (Range) %BL |
|---|---|---|
| TL (9) | 70.7±10.2 (57.1–90.9) | 172.4±9.5 (160.2–186.4) |
| SL (9) | 62.5±8.9 (49.7–79.7) | 152.4±7.8 (139.7–162.8) |
| BL (9) | 41.1±6.3 (31.9–51.7) | 100.0 |
| HL (9) | 21.2±3.9 (16.1–28.2) | 51.8±7.8 (39.6–62.7) |
| HH (9) | 20.4±2.6 (15.8–25.6) | 50.0±4.9 (42.5–59.4) |
| Orbit %HL (1) | 19.3 | 19.3 |
| BH (9) | 17.6±2.8 (13.4–22.7) | 43.0±5.0 (19.3–49.1) |
| BH2 (9) | 12.4±2.3 (9.4–16.5) | 30.3±4.7 (22.7–38.0) |
| Minb (9) | 6.1±1.1 (4.6–8.5) | 15.0±2.0 (11.2–17.7) |
| DL (7) | 27.3±3.9 (22.8–34.3) | 65.5±3.8 (60.8–71.4) |
| AL (9) | 7.8±1.8 (5.8–12.2) | 19.0±2.6 (15.6–23.7) |
| VL (5) | 9.1±2.4 (6.6–12.7) | 20.9±4.0 (16.1–24.5) |
| VH (3) | 2.0±0.5 (1.4–2.5) | 5.2±0.8 (4.5–6.1) |
| CL (9) | 9.2±2.9 (6.1–14.6) | 22.6±6.9 (13.9–35.7) |
| Asc (7) | 4.5±0.9 (3.2–5.7) | 11.2±1.7 (9.5–13.9) |
| UJ %HL (5) | 5.3±1.1 (4.4–7.0) | 25.1±1.5 (23.4–27.4) |
| LJ %HL (8) | 8.6±1.4 (7.1–10.3) | 40.1±3.8 (36.0–45.8) |
| Ped (9) | 12.2±1.4 (9.4–14.0) | 29.9±2.1 (26.2–34.2) |
| Vsp (9) | 7.9±1.0 (6.5–9.7) | 19.3±1.8 (16.7–22.1) |
| **Meristics** | | |
| Dorsal fin | XI–XIII/8–10 | |
| Anal fin | III/7–8 | |
| Pelvic fin | I/5 | |
| Pectoral fin | – | |
| Vertebrae | 28–29 (14–15+13–15) | |
| VtPtLDs | 13 | |
| Caudal fin | 6–7,8+8,4–7 | |

**Abbreviations**: **AL**, length of anal fin base; **Asc**, length of ascending arm of premaxilla; **Asp1–3**, length of anal spines 1–3; **BH**, maximum body depth; **BH2**, body depth at anal fin origin; **BL**, body length; **CL**, length of caudal fin; **DL**, length of dorsal fin base; **Dsp1–13**, length of dorsal spines 1–13; **HH**, head depth; **HL**, head length; **LJ**, length of lower jaw; **Minb**, minimum body depth; **Orbit**, diameter of orbit; **Ped**, length of caudal peduncle; **SL**, standard length; **TL**, total length; **UJ**, length of upper jaw; **VH**, length of pelvic in base; **VL**, length of pelvic fin; **VtPtLDs**, vertebra associated with last dorsal fin spine; **Vsp**, length of pelvic fin spine.

†*Baringochromis davidae* sp. nov.

(Fig. 4)

**Holotype.** 2014-WA-16(2).

**Paratypes.** Two skeletons (OCO-5b-3a/b; OCO-5-8/23(6)).

**Locality, horizon and age.** Outcrop Waril (0°40´56.21´´N 35°43´7.43´´E) in Central Kenya; Ngorora Formation, member E; late Miocene (9-10 Ma) (see Rasmussen et al., 2015).

**Diagnosis.** Distinguished from other species of the genus †*Baringochromis* by the presence of unicuspid oral teeth and a deep and stout body.

**Etymology.** Species named after Mr David Chebor, Kenya, for his keen and enthusiastic contribution to the excavation, recovery and preparation of the specimens during our fieldwork in Central Kenya.

**Comparative description.** †*Baringochromis davidae* has four tubules on the lacrimal. The inner row oral dentition is tricuspid and the outer row oral dentition is unicuspid. All other characters are as described for the genus. Measurements and meristic counts are given in Table 5.

**Comment.** †*Baringochromis davidae* shows unicuspid oral dentition, whereas the other species of †*Baringochromis* recognized here have exclusively revealed tricuspid oral dentition. †*B. davidae* cannot be assumed to represent a separate genus solely on the basis of its possession of conical teeth. Different tooth shapes in the oral jaws are known from several cichlid species of the same genus (i.e. species of *Alcolapia*, *Haplochromis*, *Ophthalmotilapia*, and *Telmatochromis*, see Van Oijen, 1996; Hanssens and Snoeks, 1999; Tichy and Seegers, 1999; Hanssens and Snoeks, 2003). Moreover, osteological, meristic and morphometric characters of †*B. davidae* are similar to those of the other species of †*Baringochromis* described above.

**Table 5. Morphometric measurements and meristic counts of the holotype and designated paratypes of *Baringochromis davidae* sp. nov.**

| Character | Mean±SD (Range) mm | Mean±SD (Range) %BL |
|---|---|---|
| TL (2) | 79.5 | 165.7±10.6 (158.2–173.2) |
| SL (2) | 71.0±6.8 (66.2–75.8) | 147.5±4.6 (144.3–150.8) |
| BL (2) | 48.1±3.1 (45.9–50.3) | 100.0 |
| HL (2) | 22.9±3.7 (20.3–25.5) | 47.4±4.6 (44.2–50.7) |
| HH (2) | 24.2±0.8 (23.7–24.8) | 50.4±1.6 (49.3–51.6) |
| Orbit | | |
| BH (2) | 22.9±1.8 (21.7–24.1) | 47.6±0.6 (47.2–48.0) |
| BH2 (2) | 19.2±0.5 (18.9–19.6) | 40.1±3.6 (37.5–42.6) |
| Minb (2) | 7.2±0.5 (6.8–7.5) | 14.9±1.9 (13.6–16.3) |
| DL (2) | 31.0±0.1 (31.0–31.1) | 64.7±4.4 (61.6–67.8) |
| AL (2) | 10.3±2.4 (8.6–12.1) | 21.7±6.4 (17.2–26.3) |
| VL (1) | 10.0 | 21.7 |
| VH (1) | 3.5 | 7.5 |
| CL (2) | 11.1±6.8 (6.3–16.0) | 23.6±15.8 (12.5–34.8) |
| Asc (2) | 5.1±0.8 (4.5–5.6) | 10.6±2.3 (9.0–12.2) |
| UJ %HL (1) | 7.2 | 35.6 |
| LJ %HL (2) | 10.9±19 (8.5–12.0 | 47.7±0.9 (47.1–48.4) |
| Ped (2) | 14.4±1.4 (13.4–15.5) | 30.0±1.1 (29.2–30.8) |
| Vsp (2) | 8.7±1.9 (7.4–10.0) | 18.3±5.0 (14.7–21.9) |
| **Meristics** | | |
| Dorsal fin | XII/10 | |
| Anal fin | III/8–9 | |
| Pelvic fin | I/5 | |
| Pectoral fin | – | |
| Vertebrae | 26–28 (12–14+13–14) | |
| VtPtLDs | 10–12 | |
| Caudal fin | 4–6,8+8,4–7 | |

**Abbreviations**: **AL**, length of anal fin base; **Asc**, length of ascending arm of premaxilla; **Asp1–3**, length of anal spines 1–3; **BH**, maximum body depth; **BH2**, body depth at anal fin origin; **BL**, body length; **CL**, length of caudal fin; **DL**, length of dorsal fin base; **Dsp1–13**, length of dorsal spines 1–13; **HH**, head depth; **HL**, head length; **LJ**, length of lower jaw; **Minb**, minimum body depth; **Orbit**, diameter of orbit; **Ped**, length of caudal peduncle; **SL**, standard length; **TL**, total length; **UJ**, length of upper jaw; **VH**, length of pelvic in base; **VL**, length of pelvic fin; **VtPtLDs**, vertebra associated with last dorsal fin spine; **Vsp**, length of pelvic fin spine.

### 4.3.3. Juvenile specimens of †*Baringochromis*

All species of †*Baringochromis* are relatively small-sized cichlids with a maximum total length of 90 mm. Studies of recent small-sized species of *Haplochromis* (maximum SL of 7.4 to 8.5 cm) have shown that individuals become fertile at a size of only 5 cm SL (Gee and Gilbert, 1965; Witte, 1981). In addition, ontogenetic studies on recent cichlids have demonstrated that the larvae are generally smaller than 1 cm and always show a prominent yolk sac, which disappears during the juvenile stage (Fujimura and Okada, 2007; Makenzie, 2013, unpublished honors thesis).

The specimens of †*Baringochromis* show a broad distribution in size, with 55 specimens displaying a standard length of less than 5 cm. The smallest specimen whose SL could be

measured is 1.4 cm long. In further 27 specimens, the SL could not be determined because the posterior outline of the hypural plates was unclear, but their mean total length is approximately 1.2 cm (see also Rasmussen et al., 2015). Even the smallest specimens show no remnants of a yolk sac (Fig.12).

We consider all specimens with SL <5 cm to represent juvenile specimens of †*Baringochromis* rather than adult dwarf species. This view is supported by the observation that the same characters are present as are seen in the adult specimens, such as tricuspid oral jaw dentition, otolith outline and number of fin spines, rays and vertebrae. Moreover, in some of the smallest specimens the fin rays are only recognizable as pale impressions, which may indicate incomplete ossification (Fig. 12). Incomplete ossification is also visible in the vertebrae (Fig. 12).


**Figure 12. Juvenile specimen of †*Baringochromis* spp. showing weakly ossified fin rays and vertebrae; lateral view** (head in dorsolateral view; OCO-5-16(1)).

### 4.3.4. Further specimens of †*Baringochromis*

233 further specimens are assigned to †*Baringochromis* sp. because of incomplete or fragmentary preservation, which precludes species identification.

## 4.4. Discussion

### 4.4.1. Systematic demarcation

The specimens described here can be assigned to the family Cichlidae based on the presence of the typical cichlid caudal skeleton bearing five hypurals, two epurals, a free uroneural, the presence of a neural arch and autogenous haemal spine but absence of a neural spine in preural vertebra 2 (PU2), and the presence of a non-autogenous haemal spine in PU3 (Fujita, 1990; Sebilia and Andreata, 1991; Fujita, 1994). Moreover, †*Baringochromis* possesses 16 (8+8) principal caudal fin rays, a pelvic fin with five soft rays and one spine, and a lateral line divided into anterior and posterior trunk canal segments separated by a gap of at least two scale rows, a feature which is also commonly found in (but is not apomorphic for) cichlids (Webb, 1990; Takahashi and Nakaya, 2002; Nelson, 2006).

Discrimination between cichlid clades is essentially based on synapomorphies related to soft tissue or delicate structures (e.g. Regan, 1920, 1922; Greenwood, 1978; Lippitsch, 1990; Stiassny, 1991; Casciotta and Arratia, 1993; Lippitsch, 1995; Kullander, 1998, 2003), which are usually not preserved or are unrecognizable in fossils.

Given their Miocene age, we assign our fossils to the African cichlids (subfamily Pseudocrenilabrinae), which diverged from the South American cichlids at least 56 My ago (Azuma et al., 2008; Murray, 2001; Friedman et al., 2013).

The presence of tricuspid inner row dentition on the oral jaws is a useful character for the discrimination of lineages within the African cichlids. This character, which is present in †*Baringochromis*, is the synapomorphy that uniquely distinguishes the Haplotilapiines (Schliewen and Stiassny, 2003), and †*Baringochromis* can therefore be interpreted as an ancient haplotilapiine cichlid.

### 4.4.2. Position of †*Baringochromis* within the Haplotilapiines

For further interpretation of the phylogenetic position of †*Baringochromis* we focus on certain osteological characters that can help to discriminate between the lineages of the Haplotilapiines.

The Etiini are the only tribe within the Haplotilapiines that display two predorsal bones (Schliewen and Stiassny, 2003). As †*Baringochromis* shows a single predorsal bone it is unlikely to represent a member of the Etiini.

Further powerful characters for the identification of haplotilapiine tribes are the number of sensory canals (= tubules) on the lacrimal and the number of infraorbitals (Trewavas, 1983; Takahashi, 2003a). According to these authors, only six of the 22 haplotilapiine lineages show the lateral line branched into four or five tubules as seen in †*Baringochromis* (Fig. 13). These tribes are the Cyprichromini, Ectodini, Haplochromini, Lamprologini, Oreochromini and Trematocarini. In light of the high informational value of lacrimal morphology in previous studies (Trewavas, 1983; Takahashi, 2003a), we consider †*Baringochromis* to be more closely related to these six tribes than to any other haplotilapiine.

### 4.4.3. Relationship of †*Baringochromis* to the Cyprichromini, Ectodini, Haplochromini, Lamprologini, Oreochromini and Trematocarini

The possession of six infraorbitals (io), as present in †*Baringochromis*, is a feature common to the Cyprichromini, Haplochromini and Oreochromini, but is not found in the Ectodini, Lamprologini or Trematocarini (Trewavas, 1983, see also Fig. 13; Takahashi, 2003a). Moreover, a short io2 with a sensory canal as seen in the fossil is present only in the Cyprichromini, Haplochromini and Oreochromini among the six tribes under discussion (Takahashi, 2003a). It is therefore likely that †*Baringochromis* is more closely related to the Cyprichromini, Haplochromini and Oreochromini than to the Ectodini, Lamprologini and Trematocarini. This interpretation is further supported by the morphology and pattern of the lateral line tubules/sensory canals on the lacrimal. With respect to this character, †*Baringochromis* is clearly distinct from the Trematocarini, which show expanded lateral line tubules that are almost in contact with each other (Fig. 13G1 and Takahashi, 2003a), and also differs clearly from the Lamprologini, because these forms have much shorter lateral line tubules than those seen in †*Baringochromis* (Fig. 13). Further evidence which suggests that †*Baringochromis* does not belong to the Lamprologini is provided by the generally trapezoidal shape of the flank scales in the latter (see Lippitsch, 1998), while †*Baringochromis* shows the more 'normal' character state in having ovate flank scales (Fig. 10), and also has fewer dorsal and anal fin spines than do members of the Lamprologini (Fig. 13).

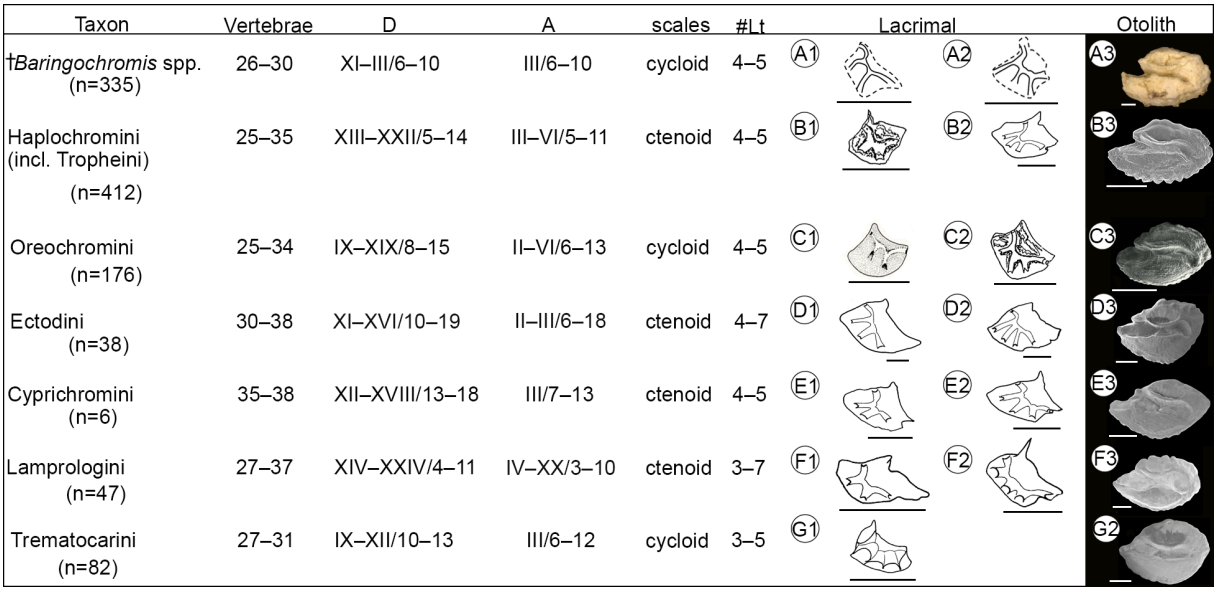


**Figure 13. Meristic counts, scale types, lacrimal morphology and otoliths of †*Baringochromis* gen. nov. and representatives of all haplotilapiine cichlids with the lateral line branched into four or five tubules** (meristic counts from this study**,** Poll (1986), and Trewavas (1983); scale type from this study and Poll (1986); lacrimal morphology from this study (A1–A2), Trewavas (1983) (C1), Cichocki (1976) (B1, C2) and Takahashi (2003a) (B2, D1–G); pictures of right otolith from this study (A3–B3, D3–G2) and Tichy and Seegers (1999) (C3)); species from which lacrimals and otoliths are illustrated are abbreviated with letters (**A1–A3**, †*Baringochromis* spp. (A1 OCO-5-37(1); A2 OCO-5-38R(5); A3 OCO-5-23R(2)); **B1**, *Pseudocrenilabrus philander*; **B2**, *Lobochilotes labiatus*; **B3**, *Tropheus duboisi* (NHM 2011/0227/0382); **C1**, *Alcolapia* (*Oreochromis*) *alcalica*; **C2**, *Oreochromis* (*Tilapia*) *mossambicus*; **C3**, *Oreochromis latilabris*; **D1**, *Xenotilapia tenuidentata*; **D2**, *Xenotilapia boulengeri;* **D3**, *Ophthalmotilapia ventralis* (ZSM 043241_(P-AA-1001)); **E1**, *Paracyprichromis brieni*; **E2**, *Cyprichromis microlepidotus*; **E3**, *Cyprichromis leptosoma* (ZSM 043238_(P-AA-0995)); **F1**, *Julidochromis ornatus*; **F2**, *Neolamprologus toae*; **F3**, *Neolamprologus tetracanthus* (NHM 2011/0227/0356); **G1–G2**, *Trematocara marginatum* (G2, ZSM 039570). Abbreviations: **A**, anal fin formula; **D**, dorsal fin formula; **#Lt**, number of lateral line tubules on the lacrimal; **n**, number of specimens. All lacrimal scale bars equal 5 mm; all otoliths scale bars equal 1 mm.

### 4.4.4. Relationship of †*Baringochromis* to the Cyprichromini, Ectodini, Haplochromini, Lamprologini, Oreochromini and Trematocarini considered in the light of otolith data

Preliminary remark: Saccular otoliths (termed otoliths in the following) are additional useful tools in taxonomic and systematic studies of teleosts (Nolf, 1985). According to Gaemers (1984, 1986), Nolf (1985) and Reichenbacher and Reichard (2014), the most informative otolith character at higher taxonomic levels (genus, tribe, family) is the sulcus morphology.

Although little information is available for cichlid otoliths, the few studies that have appeared have demonstrated their usefulness in cichlid systematics (i.e. Gaemers, 1984, 1986; Gaemers and Crapon de Crapona, 1986). We have assembled cichlid otoliths for the six tribes under discussion (i.e. those that possess a lacrimal with four or five lateral line tubules) and compared them with those of †*Baringochromis* (Figs. 11, 13). The otoliths of †*Baringochromis* are clearly distinct from those of the Trematocarini (Figs. 11G1–G2)

because the latter display an almost straight sulcus, whereas the sulcus is curved in its posterior part in †*Baringochromis*. Furthermore, the otoliths of †*Baringochromis* do not resemble those of the Lamprologini and Ectodini because the posterior part of the cauda in the Lamprologini (Figs. 11F1–F2) is much more expanded than in †*Baringochromis* and the cauda of the Ectodini (Figs. 11D1–D2) is much more curved posteriorly compared to †*Baringochromis*. It thus appears that the overall otolith and sulcus morphology of †*Baringochromis* is most similar to that of the Cyprichromini, Haplochromini and Oreochromini (Fig. 11).

### 4.4.5. Relation of †*Baringochromis* to the Cyprichromini, Haplochromini and Oreochromini

Thus, on the basis of osteological and otolith data, it can be concluded that †*Baringochromis* shows greater affinity to the Cyprichromini, Haplochromini and Oreochromini than to other haplotilapiine tribes. However, it is difficult to confidently assign †*Baringochromis* to any one of these three groups.

Cycloid scales as seen in †*Baringochromis* are present in the Oreochromini, while Cyprichromini and Haplochromini have ctenoid scales (see Lippitsch, 1990, 1992, 1995, 1998; Dunz and Schliewen, 2013) C (Fig. 13). However, it is known that cycloid scales are not only present in several basal cichlids (like the Oreochromini), but also in the more derived Perissodini, which indicates that this character can be present or absent with no indication on the phylogenetic relationships (Lippitsch, 1995, 1998; Dunz and Schliewen, 2013).

In otoliths of the Haplochromini and Oreochromini studied here, the ratio of sulcus length to ostium length is similar to that seen in †*Baringochromis* (Haplochromini: 2.12–2.19; Oreochromini: 2.13–2.14; †*Baringochromis* 2.11–2.26) and clearly exceeds the range for the Cyprichromini (1.60–1.72). In addition, among the collection of present-day otoliths, three other features of the otoliths of †*Baringochromis* are restricted to the Haplochromini: i) the high set ventral line, ii) the indentation behind the dorsal/median tip at the posterior segment and iii) the presence of a rather deep excisura (Fig. 11). The otolith data therefore indicate that †*Baringochromis* is less closely related to the Cyprichromini than to the Haplochromini and Oreochromini, which is consistent with the osteological data.

Further evidence for a closer relationship of †*Baringochromis* to Haplochromini and Oreochromini rather than to Cyprichromini is provided by meristic data. Cyprichromini have much higher numbers of vertebrae and dorsal fin rays than †*Baringochromis* (Fig. 13). In

contrast, the ranges of the meristic characters of †*Baringochromis* entirely overlap with those of the Oreochromini and Haplochromini, with the exception of the dorsal spines in the Haplochromini (Fig. 13).

### 4.4.6. Statistical analyses

We have performed a PCoA including †*Baringochromis* and all lineages (with the exception of the Etiini, which possess two predorsal bones, see above) and almost all genera of the Haplotilapiines (with exception of *Baileychromis* Poll 1986 and *Pseudosimochromis* Nelissen 1977, which were not available). The scatter plot revealed a high degree of overlap between the haplotilapiine lineages themselves and also between several haplotilapiine lineages and †*Baringochromis* (data not shown). The meristic data therefore cannot be used to discriminate between †*Baringochromis* and the haplotilapiine lineages at a higher systematic level. However, a PCoA including only the six tribes that have lacrimals with four or five tubules, clearly supports the results of our comparative study: the Lamprologini are well separated from †*Baringochromis*, †*Baringochromis* shows little overlap with the Ectodini and Trematocarini, but overlaps largely with the Haplochromini and Oreochromini (Fig. 14A). If only the Cyprichromini, Haplochromini and Oreochromini are included in the PCoA, the scatter plot separates the Cyprichromini from †*Baringochromis*, but the Haplochromini and Oreochromini still broadly overlap with it (Fig. 14B).

Taking all characters into consideration, †*Baringochromis* presents a character set that can be interpreted as a mosaic of traits found in two modern haplotilapiine tribes, i.e. the Haplochromini and Oreochromini.

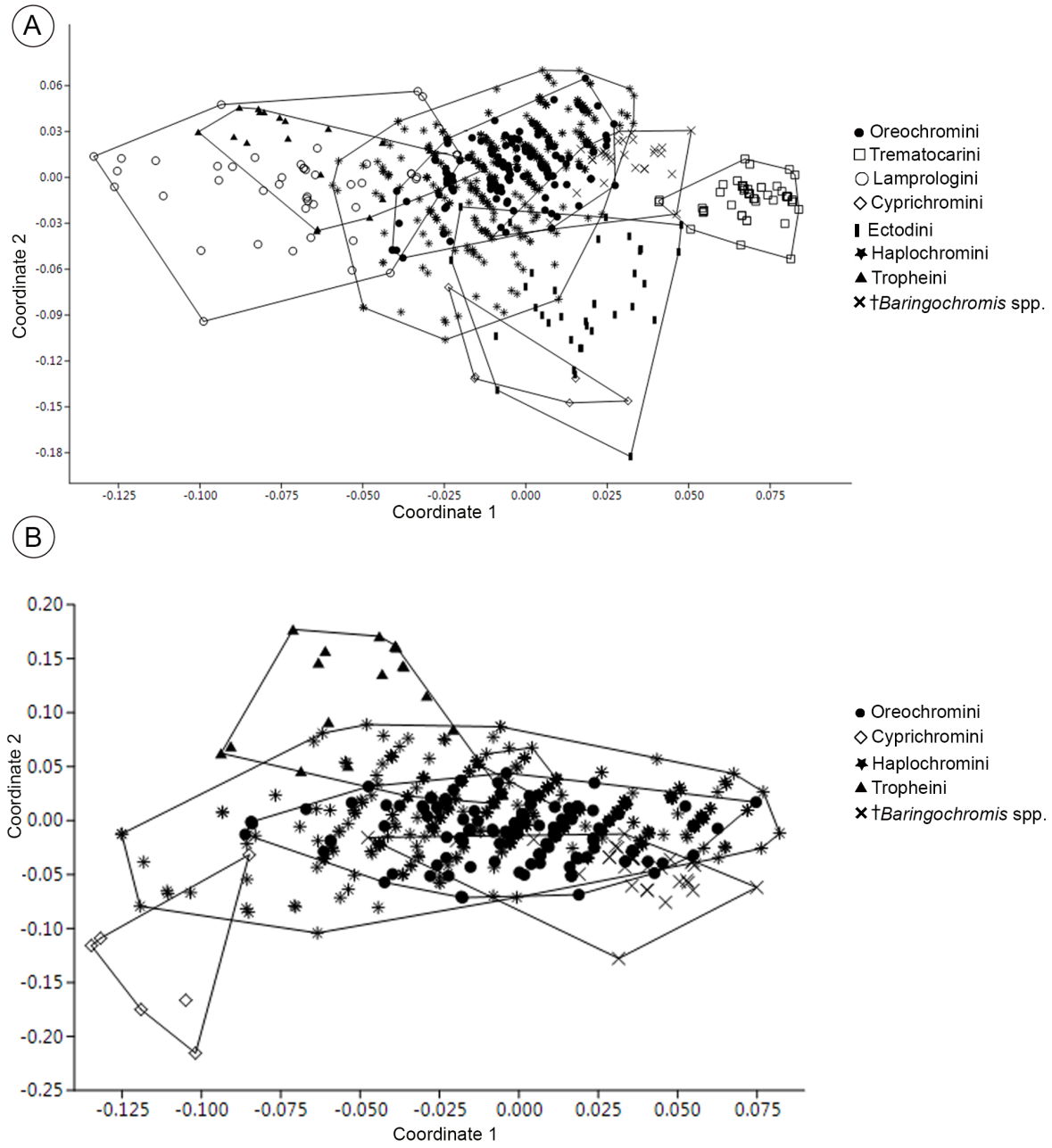


**Figure 14. Principal Coordinates Analysis (PCoA) scatter plots based on nine meristic and osteological characters. A** plot of †*Baringochromis* gen. et sp. nov. and those tribes examined in this study that possess a lacrimal with four or five tubules (761 specimens). Coordinate 1 explains 50.09% of the variation and Coordinate 2 explains 26.96% of the variation. **B** plot of †*Baringochromis* gen. et sp. nov. and the Oreochromini, Haplochromini and Cyprichromini (594 specimens). Species core limits are visualized as convex hulls. Coordinate 1 explains 45.60% of the variation and Coordinate 2 explains 25.54% of the variation.

### 4.4.7. The †*Baringochromis* species flock

The four species described in this study most probably represent a fossil species flock of four closely related sympatric species. They can be readily discriminated based on differences in head and body shape, as well as their oral tooth morphology. The variation in tooth shape is

most probably related to trophic adaptation, because cichlid species are well known to rapidly adapt to differing environmental conditions and these are commonly linked to variations in diet (Albertson et al., 2003; Kocher, 2004; Albertson and Kocher, 2006).

Closely related species that co-exist in the same lake and are distinguishable based on overall body, head and mouth shape are also known for teleost groups other than cichlids. Examples include the species of *Cyprinodon* in Laguna Chichancanab in Mexico (Strecker, 2006) and the species of *Orestias* in Lake Titicaca, Peru (Parenti, 1984). The most prominent modern species flocks among cichlids are those of Lakes Tanganyika, Malawi and Victoria in East Africa, in which several hundreds of species have evolved within an extremely short time-span (e.g. Salzburger and Meyer, 2004; Salzburger et al., 2014).

In the fossil record, evidence for species flocks is comparatively rare. The only example for fossil cichlids is the species flock of †*Mahengechromis* from the Eocene Lake Mahenge in Tanzania (Murray, 2000). This encompasses five species, which, like the species of †*Baringochromis*, are mainly separated from one another based on their head shapes (Murray, 2000).

### 4.4.8. Comparison with previously described fossil cichlids from Africa, Arabia and Europe

In order to verify the taxonomic status of †*Baringochromis*, we carried out a comparative study of all previously described fossil cichlids that exhibit at least some of the characters preserved in †*Baringochromis*. Because it is known that cichlids may show intraspecific variation in the number of anal fin spines (three or four) (see Trewavas, 1983), we have not used this character to discriminate between †*Baringochromis* and other fossil cichlids here.

The presence of more than one predorsal bone discriminates †*Macfadyena dabanensis* Van Couvering 1982, unidentified cichlids termed 'Form C' (see Van Couvering, 1982), †*Kalyptochromis*, †*Palaeofulu kuluensis* Van Couvering 1982, and ?*Heterochromis* (Lippitsch and Micklich, 1998) from †*Baringochromis*, which possesses one predorsal bone.

An elevated number of dorsal fin spines that differs from those of our fossil has been reported for †*Oreochromis lorenzoi* Carnevale et al. 2003, †*Oreochromis harrisae* Murray and Stewart 1999, †*Tilapia fossilis* and †*Tilapia melanopleura* White 1937, †*Tilapia nigra* Trewavas 1937, and †*Tilapia crassispina* Arambourg 1947.

Moreover, †*Mahengechromis* spp. Murray 2000, unidentified cichlids termed 'Form D' by Van Couvering 1982, and undetermined cichlids described by Weiler (1970) show fewer

vertebrae than †*Baringochromis*. The species of †*Palaeochromis* Sauvage 1907 can be discriminated from †*Baringochromis* based on their slightly lower number of vertebrae and their oral dentition.

Ctenoid scales are reported for †*Nderechromis cichloides* Van Couvering 1982 and undetermined cichlids termed 'Tilapiini Group3' described by Lippitsch and Micklich (1998), and these forms are therefore unlikely to be closely related to †*Baringochromis*, which displays cycloid scales.

†*Baringochromis* can be clearly separated from cf. *Tylochromis* Regan 1920 described from Lybia and Egypt by Otero et al. (2015) and Murray (2002, 2004) respectively, and undetermined cichlids termed Cichlidae 'Form D' described by Van Couvering (1982) based on its unicuspid or bicuspid pharyngeal dentition.

Based on the presence of six lateral line tubules on the lacrimal in *Pelmatochromis* sp. (Van Couvering, 1982) it appears improbable that it is closely related to †*Baringochromis*, which shows either five or four tubules on the lacrimal.

The cichlids described in open nomenclature by Van Couvering (1982) and Lippitsch and Micklich (1998) i.e. 'Form A', 'Form B', '?Tilapia Cichlidae Form A', 'Cichlidae Form B', 'Cichlidae Form A', 'Cichlidae Form B', 'Cichlidae Form C', 'Cichlidae spp. Group A', 'Cichlidae spp. Group B', Cichlidae indet., and 'Tilapiini Group2' are more difficult to separate from †*Baringochromis* because their meristic counts are not known. The last is unlikely to represent the same taxon as †*Baringochromis*, because according to Lippitsch and Micklich (1998) its dorsal fin bears scales on the soft-rayed part, which is not observed in †*Baringochromis*. Moreover, it is of Oligocene age and was recovered in Saudi Arabia. Also the much older age (Oligocene) of 'Form A' and 'Form B' (Van Couvering, 1982) separates them from our fossil material.

The remaining taxa in open nomenclature are too poorly preserved to allow for confident discrimination between them and †*Baringochromis*.

The fossil cichlid *Oreochromis* (*Sarotherodon*) *martyni* (Van Couvering, 1982), which was also recovered from the Ngorora Formation of the Tugen Hills, needs special consideration. Both †*Baringochromis* and *O.* (*Sarotherodon*) *martyni* share similar meristic counts and the presence of cycloid scales. However, the body of *O.* (*Sarotherodon*) *martyni* is usually much deeper (BD: 33.7–38.0% of SL, mean not known; see Van Couvering 1982) compared to the body of the four species of †*Baringochromis* (BD: 21.9–32.7% of SL; 27.1 ± 3.0% of SL). Moreover, given the time lag between the two fossil species (*O.* (*Sarotherodon*) *martyni*,

approximately 12 Ma (Bishop, 1971) and †*Baringochromis*, 9–10 Ma) and the marked capacity of cichlids to evolve within a short time, it is unlikely that these two fossil cichlids represent the same species.

## 4.5. Conclusion

†*Baringochromis* is the third fossil cichlid taxon to be described from the Ngorora Fish Lagerstätte. This underlines the significance of the Ngorora fish Lagerstätte in Central Kenya as a unique archive for understanding the evolutionary history of the African cichlids. Comparative analysis of osteological characters, otolith morphology and meristics using a comprehensive set of comparative material derived from all haplotilapiine lineages indicates that †*Baringochromis* has a mosaic character set intermediate between the Oreochromini and Haplochromini. The presence of a Miocene cichlid closely related to the almost pan-African tribe Oreochromini and the exclusively East African Haplochromini suggests a scenario in which widely distributed precursor lineages hybridized in rivers and lakes prior to the formation of the modern Rift Lakes. †*Baringochromis* thus confirms the previous hypothesis derived from molecular analyses, according to which riverine cichlids played a major role in the subsequent diversification of lacustrine cichlids.

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# 5. Conclusion and Outlook

The discovery of three new extinct taxa from the upper Miocene of the Central Kenya Rift adds significantly to our understanding of the evolutionary history of the two most widespread freshwater fish groups in Africa, the Cyprinodontiformes and Cichlidae. †*Kenyaichthys* constitutes the first fossil record of a member of the cyprinodontiform suborder Aplocheiloidei, while the new cichlid taxa †*Protochromis* and †*Baringochromis* most likely represent precursor lineages that contributed to the cichlid radiation in present-day Lake Tanganyika. Furthermore, the distribution of †*Protochromis* and †*Baringochromis* in the upper Miocene of the Central Kenya Rift and their inferred phylogenetic relationships with present-day Lake Tanganyika cichlids provide additional support for the previously suggested existence of an ancient East-West-directed hydrological network, and corroborate a scenario in which riverine cichlids played a major role in the diversification of the East African cichlids before the modern Rift Lakes were formed.

Notably, all three new fossil taxa possess unique mosaic-like character sets, which are not found in these combinations among their closest recent representatives. This implies that thorough analysis of fish fossils, together with a comprehensive comparative dataset derived from extant relatives, can help to reconstruct character evolution and will contribute to the correct interpretation of apomorphies, plesiomorphies or convergences. Moreover, the presence of mosaic-like character sets may indicate that the evolutionary history of the studied groups of Cyprinodontiformes and Cichlidae cannot be described simply as a sequence of lineage divergences, but was probably much more complicated, and influenced by introgression and hybridization. This hypothesis is consistent with recent phylogenetic studies based on nuclear markers for the African Cichlidae (Weiss et al. 2015).

The abundant fossil material available for this dissertation has permitted the description of two species flocks. The specimens of †*Kenyaichthys* have been suggested to represent a species flock *in statu nascendi*, due to the high variability in their osteological characters and the overlap in their meristics and morphometrics. Such species flocks are quite common in recent fishes that are characterized by different levels of reproductive isolation (e.g. Villwock, 1982; Strecker, 2006; Geiger et al., 2013). In contrast, the specimens of †*Baringochromis* document a true species flock comprising four species, distinguished by differences in head and oral tooth shapes, similar to the species of †*Mahengechromis* (Murray, 2000), which is

the only fossil species flock reported from Africa so far. This implies that species flocks may not have been as rare as the few previously reported examples in the fossil record suggest.

The numerous and well-preserved fossils described in this thesis underline the importance of fossil 'Lagerstätten' for the identification of fish fossils at genus and species level. The most remarkable feature of the Lukeino and Ngorora Formations is that they have yielded exceptionally well-preserved fish fossils in such large numbers. Such an abundance of material is rarely available for paleontological studies, and descriptions of fossil vertebrate species are often based on a few specimens (e.g. Woodward, 1939; Miller, 1948; Arratia et al., 2001; Carnevale et al., 2003; Malabarba and Malabarba, 2008; Chen et al., 2010; Carnevale et al., 2011; Gaudant, 2012, 2013; Carnevale and Collette, 2014). As a result, there is a wide gap in our knowledge of inter- and intraspecific variation in the fossil record, and species limits are not always precise. The samples of over 100 cyprinodontiform and more than 300 cichlid specimens analysed here have provided a rare opportunity to study inter- and intraspecific variation including polymorphism of certain characters. The new data suggest that levels of intraspecific variation, at least in these fossil groups of Cyprinodontiformes and Cichlidae, might be much greater than commonly assumed.

East Africa is thought to have undergone intense aridification during the Miocene (Zachos et al., 2001). Based on palynological and palaeobotanical markers, authors have found strong evidence for a transition from tropical to semiarid conditions during the middle-late Miocene in East Africa (Jacobs, 2002; Bobe, 2006; Jacobs et al., 2010; Rasmussen et al., 2015). The transition from a cichlid-dominated, tropical fauna in the middle–late Miocene Ngorora Formation (9–10 Ma) to a late Miocene (ca. 6 Ma) fish fauna dominated by eurytherm killifish agrees well with these previous assumptions.

This dissertation demonstrates that the use of a comprehensive comparative dataset is essential for a reliable phylogenetic understanding of cichlid fossils. The dataset used here included for the first time all currently recognized lineages of African cichlids. It can be assumed that the phylogenetic placement of all previously described fossil cichlids from Africa, most of which were simply assigned to either Tilapiini or Haplochromini (e.g. Trewavas, 1983), can be significantly improved based on a revision that includes the new dataset. This will undoubtedly enhance our understanding of the evolutionary history of African cichlids.

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# Appendix for chapters 3 and 4

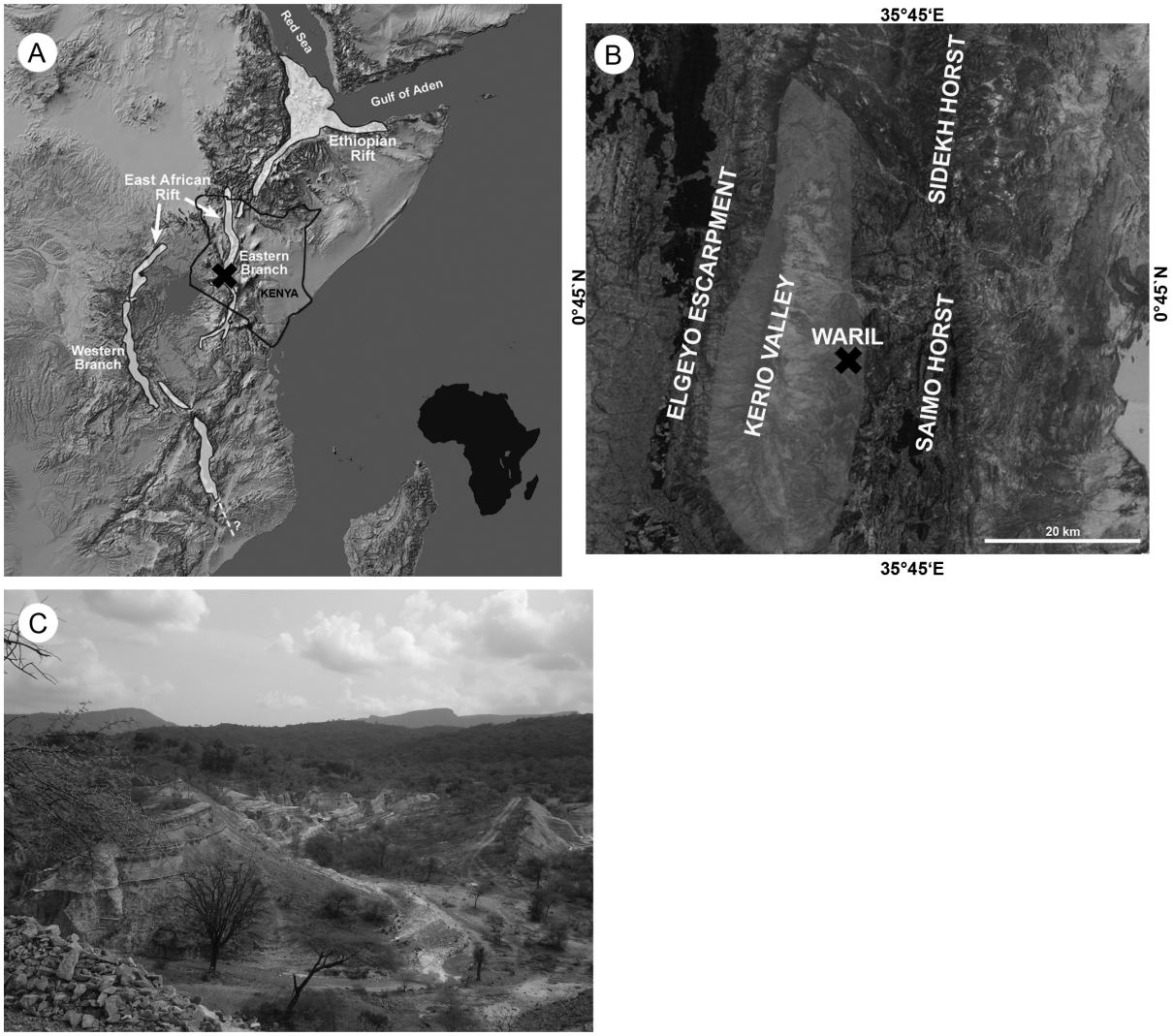


**Figure S1. Maps showing the location of the study area. A** East African Rift Valley, with the area of the Tugen Hills marked (source: http://www.makingitmagazine.net/?p=2443 and http://geology.com/articles/east-africa-rift.shtml). **B** Location of Waril to the west of the Saimo and Sidekh horsts in the Tugen Hills (source: Google Earth, Landsat). **C** Overview of the Waril site. The fossiliferous layers lie at the base of the outcrop (photo: M. Altner).

## Comparative Material

Subfamily PSEUDOCRENILABRINAE, Tribe **Boulengerochromini**: *Boulengerochromis microlepis* (Boulenger 1899) (ZSM 040843, MRAC74-06-P-18-21_1, MRAC74-06-P-18-21_2, MRAC74-06-P-18-21_3, MRAC74-06-P-18-21_4, MRAC-107323-107328_1, MRAC-107323-107328_2, MRAC-107323-107328_3, MRAC-107323-107328_4).
Tribe **Bathybatini**: *Bathybates fasciatus* Boulenger 1901 (ZSM 041479_(DRC-2011+0950) & ZSM 041479_(DRC-2011+0949), MRAC-112222-112225_1, MRAC-112222-112225_2, MRAC-112222-112225_3), *Bathybates ferox* Boulenger 1898 (MRAC-112181-112186_1, MRAC-112181-112186_2, MRAC-112181-112186_3, MRAC-112181-112186_4, MRAC-

112181-112186_5, MRAC-112181-112186_6), *Bathybates graueri* Steindachner 1911 (MRAC-112463-112467_1, MRAC-112463-112467_2, MRAC-112463-112467_3, MRAC-112463-112467_4, MRAC-112463-112467_5), *Bathybates hornii* Steindachner 1911 (MRAC-P112482-112483_1, MRAC-P112482-112483_2), *Bathybates leo* Poll 1956 (MRAC-112492-112496_1, MRAC-112492-112496_2, MRAC-112492-112496_3, MRAC-112492-112496_4), *Bathybates minor* Boulenger 1906 (MRAC-94-069-P-1025-1028_1, MRAC-94-069-P-1025-1028_2, MRAC-94-069-P-1025-1028_3, MRAC-94-069-P-1025-1028_4, MRAC-96-031-P-1238-1271_1, MRAC-96-031-P-1238-1271_2, MRAC-96-031-P-1238-1271_3, MRAC-96-031-P-1238-1271_4, MRAC-96-031-P-1238-1271_5, MRAC-96-031-P-1238-1271_6, MRAC-96-031-P-1238-1271_7, MRAC-96-031-P-1238-1271_8, MRAC-96-031-P-1238-1271_9, MRAC-96-031-P-1238-1271_10, MRAC-96-031-P-1238-1271_11, MRAC-96-031-P-1238-1271_12, MRAC-96-031-P-1238-1271_13, MRAC-96-031-P-1238-1271_14, MRAC-96-031-P-1238-1271_15, MRAC-96-031-P-1238-1271_16, MRAC-96-031-P-1238-1271_17, MRAC-96-031-P-1238-1271_18), *Bathybates vittatus* Boulenger 1914 (MRAC-P-112487-112488_1, MRAC-P-112487-112488_2), *Hemibates stenosoma* (Boulenger 1901) (MRAC-94-069-P-1032-1034_1, MRAC-94-069-P-1032-1034_2, MRAC-94-069-P-1032-1034_3, MRAC-P-112134-112135_1, MRAC-P-112134-112135_2).

Tribe **Trematocarini**: *Trematocara kufferathi* Poll 1948 (MRAC-94-069-P-1048-1096_1, MRAC-94-069-P-1048-1096_2, MRAC-94-069-P-1048-1096_3, MRAC-94-069-P-1048-1096_4, MRAC-94-069-P-1048-1096_5, MRAC-94-069-P-1048-1096_6, MRAC-94-069-P-1048-1096_7, MRAC-94-069-P-1048-1096_8, MRAC-94-069-P-1048-1096_9, MRAC-94-069-P-1048-1096_10, MRAC-94-069-P-1048-1096_11, MRAC-94-069-P-1048-1096_12, MRAC-94-069-P-1048-1096_13, MRAC-94-069-P-1048-1096_14, MRAC-94-069-P-1048-1096_15, MRAC-94-069-P-1048-1096_16, MRAC-94-069-P-1048-1096_17, MRAC-94-069-P-1048-1096_18), *Trematocara macrostoma* Poll 1952 (MRAC-B0-012-P-0526-0530_1, MRAC-B0-012-P-0526-0530_4), *Trematocara marginatum* Boulenger 1899 (ZSM 039570, MRAC-94-069-P-1739-1758_1, MRAC-94-069-P-1739-1758_2, MRAC-94-069-P-1739-1758_3, MRAC-94-069-P-1739-1758_4, MRAC-94-069-P-1739-1758_5, MRAC-94-069-P-1739-1758_7, MRAC-94-069-P-1739-1758_8, MRAC-94-069-P-1739-1758_9, MRAC-94-069-P-1739-1758_10, MRAC-94-069-P-1739-1758_11, MRAC-94-069-P-1739-1758_12, MRAC-94-069-P-1739-1758_13, MRAC-94-069-P-1739-1758_14, MRAC-94-069-P-1739-1758_15, MRAC-94-069-P-1739-1758_16, MRAC-94-069-P-1739-1758_17, MRAC-94-069-P-1739-1758_19), *Trematocara nigrifrons* Boulenger 1906 (MRAC-94-069-P-0392-0417_1, MRAC-94-069-P-0392-0417_2, MRAC-94-069-P-0392-0417_3, MRAC-94-069-P-0392-0417_4, MRAC-94-069-P-0392-0417_5, MRAC-94-069-P-0392-0417_6, MRAC-94-069-P-0392-0417_7, MRAC-94-069-P-0392-0417_8, MRAC-94-069-P-0392-0417_12, MRAC-94-069-P-0392-0417_15), *Trematocara stigmaticum* Poll 1943 (MRAC-95-089-P-0298-0322_1, MRAC-95-089-P-0298-0322_2, MRAC-95-089-P-0298-0322_3, MRAC-95-089-P-0298-0322_4, MRAC-95-089-P-0298-0322_5, MRAC-95-089-P-0298-0322_6, MRAC-95-089-P-0298-0322_7, MRAC-95-089-P-0298-0322_8, MRAC-95-089-P-0298-0322_9, MRAC-95-089-P-0298-0322_12, MRAC-95-089-P-0298-0322_13, MRAC-95-089-P-0298-0322_17, MRAC-95-089-P-0298-0322_18), *Trematocara unimaculatum* Boulenger 1901 (MRAC-94-069-P-1362-1374_1, MRAC-94-069-P-1362-1374_2, MRAC-94-069-P-1362-1374_3, MRAC-94-069-P-1362-1374_4, MRAC-94-069-P-1362-1374_5, MRAC-94-069-P-1362-1374_6, MRAC-94-069-P-1362-1374_7, MRAC-94-069-P-1362-1374_8, MRAC-94-069-P-1362-1374_9, MRAC-94-069-P-1362-1374_10, MRAC-94-069-P-1362-1374_11, MRAC-94-069-P-1362-1374_12, MRAC-94-069-P-1362-1374_13), *Trematocara variabile* Poll 1952 (MRAC-94-069-P-1810-1891_1, MRAC-94-069-P-1810-1891_2, MRAC-94-069-P-1810-1891_3, MRAC-94-069-P-1810-1891_4, MRAC-94-069-P-1810-1891_5, MRAC-94-069-P-1810-1891_6, MRAC-94-069-P-1810-1891_7, MRAC-94-069-P-

1810-1891_8, MRAC-94-069-P-1810-1891_9, MRAC-94-069-P-1810-1891_16), *Trematocara* cf. *variabile* Poll 1952 (ZSM 042334), *Trematocara zebra* De Vos, Nshombo & Thys van den Audenaerde 1996 (MRAC-96-083-P-0760-0762_1, MRAC-96-083-P-0760-0762_2, MRAC-96-083-P-0760-0762_3).
Tribe **Benthochromini**: *Benthochromis horii* Takahashi 2008 (MRAC-A7-020-P-0001-0003_1, MRAC-A7-020-P-0001-0003_2, MRAC-A7-020-P-0001-0003_3), *Benthochromis melanoides* (Poll 1984) (MRAC-112548), *Benthochromis tricoti* (Poll 1948) (ZSM 040833, ZSM 043239_(P-AA-0997), ZSM 043239_(P-AA-0998), MRAC-94-069-P-1573-1578_1, MRAC-94-069-P-1573-1578_2, MRAC-94-069-P-1573-1578_3, MRAC-94-069-P-1573-1578_4, MRAC-94-069-P-1573-1578_5, MRAC-94-069-P-1573-1578_6).
Tribe **Cyprichromini**: *Cyprichromis leptosoma* "Kigoma" (Boulenger 1898) (ZSM 043238_(P-AA-0995) & ZSM 043238_(P-AA-0996), *Cyprichromis microlepidotus* (Poll 1956) (ZSM 040807), *Cyprichromis zonatus* Takahashi, Hori & Nakaya 2002 (ZSM 040812), *Cyprichromis zonatus* "Chitua" Takahashi, Hori & Nakaya 2002 (ZSM 042935), *Paracyprichromis nigripinnis* (Boulenger 1091) (ZSM 040793).
Tribe **Perissodini**: *Haplotaxodon microlepis* Boulenger 1906 (ZSM 040840, ZSM 040839, ZSM 043243_(P-AA-1005), ZSM 043243_(P-AA-1006)), *Perissodus microlepis* Boulenger 1898 (ZSM 042332 & ZSM 042331), *Plecodus multidentatus* Poll 1952 (MRAC-95-098-P-0266-0267_1, MRAC-95-098-P-0266-0267_2), *Xenochromis hecqui* Boulenger 1899 (MRAC-112569-112572-1, MRAC-112569-112572-2).
Tribe **Cyphotilapiini**: *Cyphotilapia* sp. (ZSM 043240_(P-AA-0999) & ZSM 043240_(P-AA-1000)), *Cyphotilapia* sp. (ZSM 040818), *Cyphotilapia* sp. (ZSM 040815), *Trematochromis benthicola* (Matthes 1962) (MRAC-96-083-P-0764-0772_1, MRAC-96-083-P-0764-0772_2, MRAC-96-083-P-0764-0772_3, MRAC-96-083-P-0764-0772_4, MRAC-96-083-P-0764-0772_5, MRAC-96-083-P-0764-0772_6, MRAC-96-083-P-0764-0772_7, MRAC-96-083-P-0764-0772_8, MRAC-96-083-P-0764-0772_9, ZSM 043236).
Tribe **Limnochromini**: *Gnathochromis permaxillaris* (David 1936) (ZSM 040819), *Greenwoodochromis bellcrossi* (Poll 1976) (ZSM 042335), *Limnochromis auritus* (Boulenger 1901) (ZSM 043242_(P-AA-1003) & ZSM 043242_(P-AA-1004)), *Limnochromis staneri* Poll 1949 (ZSM 040846), *Reganochromis calliurus* (Boulenger 1901) (ZSM 040799), *Tangachromis dhanisi* (Poll 1949) (MRAC-107296-107300_1, MRAC-107296-107300_2, MRAC-107296-107300_3, MRAC-107296-107300_4), *Triglachromis otostigma* (Regan 1920) (ZSM 024837_1, ZSM 024837_2, ZSM 040847).
Tribe **Ectodini**: *Asprotilapia leptura* Boulenger 1901 (MRAC-92-081-P-2250-2253_1, MRAC-92-081-P-2250-2253_2, MRAC-92-081-P-2250-2253_3, MRAC-92-081-P-2250-2253_4), *Aulonocranus dewindti* (Boulenger 1899) (MRAC-81-062-P-0066-0074_1, MRAC-81-062-P-0066-0074_2, MRAC-81-062-P-0066-0074_3, MRAC-81-062-P-0066-0074_4, MRAC-81-062-P-0066-0074_5, MRAC-81-062-P-0066-0074_6, MRAC-81-062-P-0066-0074_7, MRAC-81-062-P-0066-0074_8, MRAC-81-062-P-0066-0074_9), *Callochromis macrops* (Boulenger 1898) (ZSM 040823), *Callochromis melanostigma* (Boulenger 1906) (ZSM 042931, ZSM 024765), *Callochromis pleurospilus* (Boulenger 1906) (ZSM 024764), *Callochromis stappersii* (Boulenger 1914) (ZSM 040870, *Cardiopharynx schoutedeni* Poll 1942 (MRAC-94-069-P-1581-1591_1, MRAC-94-069-P-1581-1591_2, MRAC-94-069-P-1581-1591_3, MRAC-94-069-P-1581-1591_4, MRAC-94-069-P-1581-1591_5), *Cunningtonia longiventralis* Boulenger 1906 (ZSM 040848), *Cyathopharynx furcifer* (Boulenger 1898) (ZSM 040811), *Ectodus descampsi* Boulenger 1898 (ZSM 040810), *Grammatotria lemairii* Boulenger 1899 (ZSM 040826), *Lestradea perspicax* Poll 1943 (MRAC-P42674-42677_1, MRAC-P42674-42677_2, MRAC-P42674-42677_3, MRAC-P42674-42677_4), *Ophthalmotilapia ventralis* (Boulenger 1898) (ZSM 043241_(P-AA-1001) & ZSM 043241_(P-AA-1002)), *Xenotilapia flavipinnis* Poll 1985 (ZSM 040836), *Xenotilapia melanogenys* (Boulenger 1898) (ZSM 040813), *Xenotilapia ornatipinnis* Boulenger 1901

(ZSM 040834), *Xenotilapia rotundiventralis* (Takahashi, Yanagisawa & Nakaya 1997) (ZSM 042934), *Xenotilapia sima* Boulenger 1899 (BMNH 1961.11.22.222-224_1, BMNH 1961.11.22.222-224_2, BMNH 1994.11.3.65-68_1, BMNH 1994.11.3.65-68_2, BMNH 1899.11.27.103_1 Syntype, BMNH 1899.11.27.103_2 Syntype), *Xenotilapia spiloptera* Poll & Stewart 1975 (ZSM 040838).
Tribe **Lamprologini**: *Altolamprologus calvus* (Poll 1978) (ZSM 040877), *Altolamprologus, compressiceps* (Boulenger 1898) (ZSM 040871), *Chalinochromis brichardi* Poll 1974 (ZSM 040879), *Chalinochromis popelini* Brichard 1989 (ZSM 040820), *Chalinochromis* sp. "Ndobhoi" (ZSM 040878), *Julidochromis marlieri* Poll 1956 (ZSM 040805), *Julidochromis ornatus* Boulenger 1898 (ZSM 040788), *Julidochromis regani* Poll 1942 (ZSM 040806), *Lamprologus lemairii* Boulenger 1899 (ZSM 024240_1 & ZSM 024240_2), *Lamprologus mocquardi* Pellegrin 1903 (ZSM 038370_1 & ZSM 038370_2), *Lamprologus* sp. (ZSM 038102_(DRC-2008+435) & ZSM 038102_(DRC-2008+0437)), *Lamprologus speciosus* Büscher 1991 (ZSM 027972_1 & ZSM 027972_2), *Lamprologus teugelsi* Schelly & Stiassny 2004 (ZSM 038141_(DRC-2008+0150) & ZSM 038141_(DRC-2008+0151)), *Lepidiolamprologus cunningtoni* (Boulenger 1906) (ZSM 024336), *Lepidiolamprologus elongatus* (Boulenger 1898) (ZSM 024239_1, ZSM 024239_2, ZSM 024239_4), *Lepidiolamprologus nkambae* (Staeck 1978) (ZSM 040795), *Neolamprologus brichardi* (Poll 1974) (ZSM 033918_1, ZSM 033918_2, ZSM 040802), *Neolamprologus calliurus* (Boulenger 1906) (ZSM 040824), *Neolamprologus* sp. (ZSM 040800), *Neolamprologus nigriventris* Büscher 1992 (ZSM 028413_3 & ZSM 028413_4), *Neolamprologus pectoralis* Büscher 1991 (ZSM 028095), *Neolamprologus prochilus* (Bailey & Stewart 1977) (ZSM 040924), *Neolamprologus similis* Büscher 1992 (ZSM 028383), *Neolamprologus toae* (Poll 1949) (MRAC-94-069-P-0779-0787_1, MRAC-94-069-P-0779-0787_2, MRAC-94-069-P-0779-0787_3, MRAC-94-069-P-0779-0787_4, MRAC-94-069-P-0779-0787_5, MRAC-94-069-P-0779-0787_6, MRAC-94-069-P-0779-0787_7, MRAC-94-069-P-0779-0787_9), *Telmatochromis dhonti* (Boulenger 1919) (ZSM 024200), *Telmatochromis* sp. (ZSM 040829), *Telmatochromis temporalis* Boulenger 1898 (ZSM 024984, ZSM 024237_1, ZSM 024237_2), *Variabilichromis moorii* (Boulenger 1898) (ZSM 040832).
Tribe **Eretmodini**: *Eretmodus cyanostictus* Boulenger 1898 (ZSM 040841, ZSM 024172_1, ZSM 024172_2, ZSM 024175_1, ZSM 024175_2, ZSM 024175_3, ZSM 043237_(P-AA-0993), ZSM 043237_(P-AA-0994)), *Spathodus erythrodon* Boulenger 1900 (ZSM 040844), *Spathodus marlieri* Poll 1950 (ZSM 040842), *Tanganicodus irsacae* Poll 1950 (ZSM 042333).
**Orthochromines**: *Orthochromis kalungwishiensis* (Greenwood & Kullander 1994) (ZSM 041431_1, ZSM 041431_2, ZSM 041431_3, ZSM 041431_4, ZSM 041431_5, ZSM 041431_7, ZSM 041427), *Orthochromis kasuluensis* De Vos & Seegers 1998 (ZSM 041455_1, ZSM 041455_2, ZSM 041455_3, ZSM 041455_4, ZSM 041455_5), *Orthochromis luichensis* De Vos & Seegers 1998 (ZSM 041445_(DRC-2011+1025), ZSM 041445_2, ZSM 041445_3, ZSM 041445_4, ZSM 041445_(DRC-2011+1026), ZSM 041445_6, ZSM 041445_7), *Orthochromis luongoensis* (Greenwood & Kullander 1994) (CU 91747, ZSM 041437_1, ZSM 041437_2, ZSM 041437_3, ZSM 041437_4, ZSM 041437_5, ZSM 041437_6, ZSM 044432_6998), *Orthochromis machadoi* (Poll 1967) (BMNH 1984.2.6.104-108_1, BMNH 1984.2.6.104-108_2, BMNH 1984.2.6.104-108_3, BMNH 1984.2.6.104-108_4, BMNH 1984.2.6.104-108_5, BMNH 1984.2.6.109, BMNH 1984.2.6.113, BMNH 1984.2.6.116-131_2, BMNH 1984.2.6.116-131_3, BMNH 1984.2.6.116-131_4, BMNH 1984.2.6.132-141_1, BMNH 1984.2.6.132-141_2, BMNH 1984.2.6.132-141_3, BMNH 1984.2.6.142-145_1, BMNH 1984.2.6.142-145_2, BMNH 1984.2.6.142-145_3, BMNH 1984.2.6.142-145_4), *Orthochromis malagaraziensis* (David 1937) (ZSM 041469_(DRC-2011+1030) & ZSM 41469_(DRC-2011+1029)), *Orthochromis rubrolabialis* De Vos & Seegers 1998 (ZSM 041463_1, ZSM 041463_2, ZSM 041463_3, ZSM 041463_4, ZSM

041463_5, ZSM 041463_6, ZSM 041463_7, ZSM 041463_8), *Orthochromis* sp. (ZSM 038382, ZSM 040703_1, ZSM 040703_2), *Orthochromis* sp. Igamba (ZSM 041563_(P-AA-1077), ZSM 041563_(P-AA-1078, ZSM 041563_(P-AA-1079), ZSM 041563_1), *Orthochromis* sp. Kashinsa (ZSM 041443_1, ZSM 041443_2, ZSM 041443_3, ZSM 041443_4, ZSM 041443_5, ZSM 041429_1, ZSM 041429_2, ZSM 041429_3, ZSM 041429_4, ZSM 041429_5, ZSM 041429_6, ZSM 041429_7, ZSM 041429_8, ZSM 041429_9, ZSM 041429_10, ZSM 041429_11), *Orthochromis* sp. Mambilima (ZSM 042322_(P-AA-0698), ZSM 042322_(P-AA-0701), ZSM 041450_1, ZSM 041450_2, ZSM 041450_3, ZSM 041450_4, ZSM 041450_5, ZSM 041450_6, ZSM 041450_7, ZSM uncatalogued specimen), *Orthochromis stormsi* (Boulenger 1902) (ZSM 032359_1, ZSM 032359_2 ZSM 042319, ZSM 032410_1, ZSM 032410_2, ZSM 023693, ZSM 032393_1, ZSM 032393_2, ZSM 032393_3, ZSM 032393_4, ZSM 032393_5, ZSM 037541_1, ZSM 037541_2, ZSM 037541_3, ZSM 037603, ZSM 038337, ZSM 038129_1, ZSM 038129_2, ZSM 038129_3, ZSM 042319_(P-AA-0708)), *Orthochromis torrenticola* (Thys van den Audenaerde 1963) (ZSM 038201_(Uli-LUB-2008+008), ZSM 038201_2, ZSM 038201_4, ZSM 038201_5), *Orthochromis uvinzae* De Vos & Seegers 1998 (ZSM 041564_1, ZSM 041564_2, ZSM 041564_3, ZSM 041564_4, ZSM 041564_5, ZSM 041562_1, ZSM 041562_2, ZSM 041562_3, ZSM 041562_4, ZSM 041562_5, ZSM 041430_1, ZSM 041430_2, ZSM 041430_3, ZSM 041430_4, ZSM 041430_5, ZSM 041430_6, ZSM 041430_7).

Tribe **Haplochromini (Tropheini)**: *Astatoreochromis alluaudi* Pellegrin 1904 (ZSM 041133_2), ZSM 041146_5932, ZSM 041017_5877), *Gnathochromis pfefferi* (Boulenger 1898) (ZSM 024242 & ZSM 040923), *Haplochromis horei* (Günther 1894) (ZSM 024846_1), *Haplochromis vanheusdeni* Schedel, Friel & Schliewen 2014 (ZSM 042320, ZSM 042311, ZSM 041559_1, ZSM 041559_2, ZSM 041559_3, ZSM 041559_4, ZSM 041559_5, ZSM 041559_6, ZSM 041559_7, ZSM 041559_8, ZSM 041559_9, ZSM 041559_10, ZSM 041559_11, ZSM 041440_1, ZSM 041440_2, ZSM 041440_3, ZSM 043134), *Limnotilapia dardennii* (Boulenger 1899) (ZSM 024944_1 & ZSM 040925), *Lobochilotes labiatus* (Boulenger 1898) (ZSM 040922 & ZSM 024174_1), *Petrochromis trewavasae* Poll 1948 (ZSM 040831), *Simochromis babaulti* Pellegrin 1927 (ZSM 040927), *Simochromis diagramma* (Günther 1894) (ZSM 040926), *Tropheus duboisi* Marlier 1959 (ZSM 040792), *Tropheus moorii* Boulenger 1898 (ZSM 024982_1, ZSM 024982_2, ZSM 024982_3, ZSM 024339_1, ZSM 024339_2, ZSM 024339_3, ZSM 040828), *Tropheus polli* Axelrod 1977 (ZSM 040790).

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